EFFECT OF PHOTOPERIOD ON THE ADAPTATION OF CHICKPEA (*CICER ARIETINUM* L.) TO THE CANADIAN PRAIRIES

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By

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Abstract

Chickpea (*Cicer arietinum* L.) was recently introduced to the Canadian prairies, a region which has a short growing season in which crop maturation often occurs under cool and wet conditions. To improve the yield of chickpea, crop duration must closely match the available growing season. The objectives of this study were to: i) examine the days to flowering of diverse chickpea accessions grown in either long or short-days; ii) examine the days to flowering of selected chickpea accessions grown in a range of thermal regimes combined with either long or short days and to examine the interaction between photoperiod and day and night temperatures on crop duration; iii) determine the timing and duration of the photoperiod-sensitive phase in selected chickpea accessions, and vi) determine the genetic basis of the association between flowering time and reaction to ascochyta blight in chickpea.

A wide variation was observed in chickpea accessions for their response to flowering under long (16/8 hours day /night) and short days (10/14 hours day/night). Earlier flowering was observed under long photoperiod regimes compared with the short photoperiod regimes. Variability was detected among chickpea accessions for their flowering responses when different temperatures were combined with different photoperiods. Earlier flowering was observed under long days (16/8 hours day/night) coupled with high to moderate temperature regimes (24/16 ºC and 20/12 ºC, day and night respectively) compared to short-days (10/14 hours day and night) and moderate to low temperature regimes (20/12 ºC and 16/8 ºC day and night, respectively). Those chickpea accessions such as ICC 6821 and ICCV 96029 which originated from the lower latitudes of Ethiopia and India, respectively, flowered earlier compared to accessions such as CDC Corinne and CDC Frontier which originated from the higher latitudes and cooler temperate environments of western Canada. Photoperiod sensitivity phases were detected in chickpea

accessions adapted to the cold environments of western Canada, whereas no photoperiod sensitivity phase was identified in the extra-early flowering cultivar ICCV 96029. The duration of the photoperiod sensitive phase in the chickpea accessions was longer under short days compared to long days.

Field and growth chamber evaluation of a chickpea RIL population (CP-RIL-1) revealed the presence of variability among the lines and the two parents for their days to flowering and level of resistance to ascochyta blight. Broad sense heritability across different site-years for days to flower 0.45 to 0.78, plant height 0.48 to 0.78, ascochyta blight resistance 0.14 to 0.68, days to maturity 0.26, photoperiod sensitivity 0.83 and nodes number of first flowering 0.37 to 0.75 were estimated. Days to flower and photoperiod sensitivity were significantly r = -0.21 to -0.58 ($P \leq 0.05$ to 0.001) and -0.28 to -0.41 ($P \leq$ 0.01 to 0.001), respectively and negatively correlated with ascochyta blight resistance in the CP-RIL-1 population.

A genetic linkage map consisting of eight linkage groups was developed using 349 SNP markers. Seven QTLs were identified for days to flowering under growth chamber and field conditions on chromosomes 3, 5, 6 and 8 each and 3 QTLs on chromosome 4. The total phenotypic variation explained by QTLs for days to flowering ranged from 7 to 44%. Two QTLs for days to maturity were identified on chromosomes 3 and 8. Three QTLs, one each on chromosomes 3, 4 and 5 were identified for photoperiod sensitivity. The total phenotypic variation explained by each QTL for photoperiod sensitivity ranged from 7 to 41%. A total of three QTL for node of first flowering, one on chromosomes 3 and 8 each, and two on chromosome 4 were identified. The two QTL on chromosome 4 explained total phenotypic variations of 11 and 32%, respectively. Ten QTLs distributed across all chromosomes, except chromosomes 2 and 5, were identified for ascochyta blight resistance. The phenotypic variability explained by each QTL for

ascochyta blight resistance ranged from 7 to 17%. The molecular markers associated with these QTLs have potential for use in chickpea breeding.

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Abbreviations and Acronyms

Ab - Ascochyta blight

CDC- Crop Development Centre, University of Saskatchewan

CRD-Completely Randomized Design

cM - Centimorgans

DTF- Days to flower

DTM-Days to maturity

ICRISAT- International Crops Research Institute for the Semi-Arid Tropics

ICARDA - International Center for Agricultural Research in the Dry Areas

H^2- Broad sense heritability

LD – Long-days

LG - Linkage Group

LOD - Logarithm of Odds

MAS - Marker Assisted Selection

PVE- Proportion of Explained Phenotypic Variance

SD – Short- days

TU- Thermal units

PLHT - Plant height

PhtU-Photothermal units

QTL - Quantitative Trait Locus

RAD - Restriction-site Associated DNA

RCBD - Randomized complete block design

RIL - Recombinant Inbred Line

SAT - Semi-Arid Tropics

SNP- Single Nucleotide Polymorphism

SPG - Saskatchewan Pulse Growers

WANA - West Asia and North Africa

Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinated, diploid (2n=2x=16) plant and the second most important grain legume crop of the world after dry bean (*Phaseolus vulgaris* L.) in terms of area and volume of production. Chickpea is grown throughout a wide geographical range and in various cropping systems (Roberts et al., 1985; FAOSTAT, 2013; Singh, 1997). It is cultivated on a large area in arid and semiarid environments and has important uses as food, feed and fodder (Singh 1997; Singh and Reddy, 1991). Chickpea is a staple protein crop on the Indian sub-continent, the Near East and across the Mediterranean basin (Ladizinsky and Adler, 1976; Singh 1997). Expansion of area under chickpea cultivation has recently occurred in the USA, Canada and Australia due to its ability to fix atmospheric nitrogen in symbiosis with *Rhizobium leguminosarum* subsp. *ciceri* strains and its economic attractiveness compared to cereal crops (Croser et al., 2003; Warkentin et al., 2003; Cutforth et al., 2007). Chickpea crop residue provides a substantial amount of nitrogen for the succeeding crops, and thus restores long-term fertility and maintains the ecosystem on a sustainable basis (Aslam et al., 2003).

Canada is the second largest producer of pulse crops (4.7 million tonnes) after India (16.3 million tonnes) (Andrews and Hodge, 2010; FAOSTAT, 2013). Field production of chickpea in Saskatchewan started in the late 1990s (Knights et al., 2007). Since then, the area under chickpea production has increased in western Canada (FAOSTAT, 2013). The increased production of pulse crops on the Canadian prairies has occurred at the expense of fallow (Andrews and Hodge, 2010). A few of the key factors in the successful expansion of the chickpea industry in Canada are perseverance in the research community to develop early-maturing, ascochyta blight resistant varieties, and the willingness of Saskatchewan producers to take on a new crop and supply many markets in

light of drought and disease problems of traditional chickpea suppliers, such as Turkey and Australia.

Chickpea is an indeterminate crop; vegetative and reproductive growth often continues during the fall season when favorable environmental conditions prevail, especially when moisture is available. Under these growing conditions, the plant continues to grow vegetatively without setting pods or only filling few pods (Liu et al., 2003; Davies et al., 1999). The competition between the vegetative and reproductive growth in chickpea is considered a major yield determinant (Bonfil and Pinthus, 1995). In areas with a short growing season, excessive vegetative growth is common when moderate rainfall occurs in late summer or early fall exposing the crop to killing frost before maturity (Gaur et al., 2008; Gan et al., 2009). Longer crop duration also prolongs the exposure of the crop to biotic stresses such as ascochyta blight (Bonfil et al., 2006).

Chickpea maturation often occurs under unfavorable environmental conditions such as cool and wet in temperate environments or warm and dry in semi-arid tropics (Liu et al., 2003). Time available for chickpea to produce adequate vegetative growth and grain yield is often limited by warm or cold temperatures, rainfall distribution and biotic stresses. To achieve optimum yield, crop duration must closely match the available growing season. The chickpea breeding program at the University of Saskatchewan has a major focus on development of chickpea cultivars that mature early and are resistant to ascochyta blight, with large seed size for export markets. Environmental factors including photoperiod and mean temperature determine timing of flowering in chickpea genotypes. In addition to these environmental factors, the timing of flowering has long been reported to be influenced significantly by quantitative responses to vernalization (Saxena and Siddique, 1980). Thus, understanding of the environmental factors as well as the genetic control of flowering time in chickpea is a key factor in order to develop a strategy for improvement

of the crop adaptation to the short growing environments. In western Canada there is a considerable potential for yield increase in chickpea provided that phenology is better adapted for the short growing season environments (Miller et al., 2002; Liu et al., 2003; Warkentin et al., 2003).

Substantial efforts in chickpea improvement have been made at the International Center for Agricultural Research in the Dry Area (ICARDA) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Kumar and Abbo, 2001; Serraj et al., 2005). However, overall chickpea research has been relatively neglected due to the crop's relatively minor economic significance to most developed nations until recently when the area under production expanded in developed countries like Australia, Canada and USA. Improvement of chickpea for higher yield potential and resistance to biotic and abiotic stresses depends on the identification of allelic variation at key loci (Abbo et al., 2003). Knowledge of the genetic control and genomic locations of yield related traits would speed up the development of more productive chickpea cultivars (Cobos et al., 2007). Genomic regions underlying ascochyta blight resistance have been identified with the aid of gene-based markers (Stephens et al., 2014). These genetic markers flanking the regions could contribute to the development of ascochyta blight tolerant chickpea genotypes. Construction of high density genetic maps from gene-based markers provided an important opportunity to identify genes directly related to agronomic traits (Choudhary et al., 2012; Deokar et al. 2014). Chickpea breeding in western Canada could exploit available means to develop ascochyta blight resistance, high yielding and early maturing varieties.

Flowering time in chickpea is modulated by genotype, temperature and photoperiod (Roberts et al., 1985). The manipulation of the genetic control of flowering time in chickpea is difficult without understanding individual effects of genes governing

this trait, interaction among them, and their response to variation in temperature and photoperiod. There were three hyphotheses in this study as follow: 1) flowering time in chickpea is under genetic control and modulated by environmental conditions, 2) a photoperiod-sensitive phase exists in chickpea, and 3) earliness and ascochyta blight susceptibility are positively correlated in chickpea.

The objectives of this study were as follows.

1. To examine the days to flowering of diverse chickpea accessions grown with either long or short days.
2. To examine the days to flowering of selected chickpea accessions grown in a range of thermal regimes combined with either long or short days, and to examine the effect of the interaction between photoperiod and day and night temperatures on flowering response.
3. To determine the timing and duration of the photoperiod-sensitive phase in selected chickpea accessions.
4. To determine the genetic basis of the association between flowering time and reaction to ascochyta blight in chickpea.

The study is divided into four chapters:

In study 1, flowering response of one hundred diverse chickpea accessions to long-days and short-day photoperiods was determined under growth chamber conditions. Variability among the accessions for their response to thermal units and photo-thermal units was also detected.

Study 2 was conducted to examine the flowering response of selected chickpea accessions grown in a range of thermal regimes combined with either long or short days and to examine the interaction between photoperiod and day and night temperatures. In this

study, flowering response of eight diverse chickpea accessions to a combination of two photoperiods and three temperature regimes was evaluated under growth chamber conditions. The flowering response of the accessions to cumulative thermal and photo-thermal units was also determined.

Study 3 was designed to determine the timing and duration of the photoperiod-sensitive phase in selected chickpea accessions representative of different maturity classes, and to establish whether photoperiod sensitivity ends at floral initiation or if it extends into the phases of flower development. In this study, photoperiod sensitive and photoperiod insensitive phases in eight diverse chickpea accessions were determined by reciprocally transferring the plants from long day chambers to short day chambers. Variability in the duration of the photoperiod sensitive phase and photoperiod insensitive phase was detected.

Study 4 was conducted to determine the genetic basis of the association between flowering time and resistance to ascochyta blight in chickpea, and to map the chromosome regions that control flowering time, days to maturity, photoperiod insensitivity and resistance to ascochyta blight. Wide variability among the RILs was detected for time to flowering, ascochyta blight resistance and photoperiod sensitivity. These studies are presented in manuscript format in Chapters 3 to 6.

2. Literature Review

2.1 Origin and distribution of chickpea

Chickpea (*Cicer arietinum* L.) is a self-pollinated, diploid crop with 2n=2x=16 chromosomes and a genome size of approximately 740 Mbp (Arumuganathan and Earle 1991). Chickpea is the second most important grain legume crop of the world in terms of total production and area under harvest after dry bean (*Phaseolus vulgaris* L.) (FAOSTAT, 2013). The Fertile Crescent, the current south-eastern Turkey, and neighbouring areas of Turkey have been proposed as the center of origin for the cultivated chickpea (Van der Maesen, 1972; Singh, 1997; Lev-Yadun, et al., 2000). Based on botanical, genetic and archeological evidence, chickpea was first domesticated in these regions before the late Neolithic period (Tanno and Willcox, 2006). Phylogenetic analysis in the genus *Cicer* and cultivated chickpea using RAPD and ISSR markers revealed nonexistence of a relationship between geographic origin and the clustering of the different accessions suggesting that chickpea may have been spread by humans during different historical periods from its centre of origin in the Near East (Iruela et al., 2002). The existence of the wild relatives, particularly *Cicer cuneatum*, indicated that Ethiopia is the secondary center of genetic diversity for chickpea (Van der Maesen, 1972).

2.1.1 Market classes of chickpea

Although a narrow genetic base in cultivated gene pools of chickpea was reported (Abbo et al., 2003; Varshney et al., 2012), a substantial diversity exists in many morphological characters (Upadhaya et al., 2011a). Chickpea is mainly divided into two broad market classes, kabuli and desi, based on size, shape and seed coat colour (Van der Maesen, 1972; Muehlbauer and Rajesh, 2008). Kabuli types generally have large seeds that weigh more than 26 grams per 100 seeds, are ram-head to round in shape and have white or cream coloured seed coats. Desi types generally have seeds that are less than 26 grams

per 100 seeds and are angular in appearance with varying seed coat colour from light tan to black (Van der Maesen, 1972).

Cultivation of the desi type is centered on the Indian sub-continent and the east African region, which accounts for about three-quarters of the world production. In the Indian sub-continent chickpea is typically sown in October/November and in Ethiopia in August/September onward to January as an autumn-sown winter crop on conserved moisture during the post rainy season of the year (Bejiga, 1972; Van der Maesen, 1972). In these regions, the growing season is characterized by end-of-season drought which coincides with flowering and pod setting and also a shortening of photoperiod. The kabuli type is grown in extensive and ecologically diverse regions, extending westwards from Afghanistan through the Middle and Near East and into North Africa and southern Europe. In these regions, chickpea is sown in February to May as a spring crop coinciding with warming temperatures and the cessation of winter rains (Roberts et al., 1985; Sheldrake and Saxena, 1979).

2.1.2 Yield potential of chickpea

Chickpea is grown over a wide geographical range and in various cropping systems where prevailing diseases and pests reduce grain yield (Van der Maesen, 1972; Croser et al. 2003; Singh, 1997). Chickpea is traditionally grown as a low input crop under depleting soil moisture status with minimum input and crop management. Unlike the higher seed yield, i.e., more than 5 t/ha from large plots in the subtropical and up to 3 t/ha in irrigated plots in tropical regions, the mean global seed yield of around 0.8 t/ha shows that most farmers do not obtain high productivity because of several production constraints (Saxena, 1990). Based on the current yield gaps and potential productivity of the crop, it is very important to identify major production constraints and improve production and

productivity of chickpea around the world to meet the demand of the growing world population.

The global area under chickpea production in 2013 was about 13.5 Mha with a total production of 13.1 Mt and average productivity of 9.7 tonnes/ha (FAOSTAT 2013). Asia comprises 87% of the global chickpea production and 90% of the area (FAOSTAT, 2013). India comprises 8.3 Mha, accounting for 68% of the total global area of production and 5.89 Mt of production. Pakistan has 10% of the global area of production, 1.06 Mha of area and 0.6 Mt of production. Other countries comprise the remaining portion of production, Iran (0.54 Mha), Turkey (0.50 Mha), Myanmar (0.22 Mha), Australia (0.30 Mha), Ethiopia (0.21 Mha), Canada (0.11 Mha), Mexico (0.10 Mha), Malawi (0.09), and Syria (0.07 Mha) (FAOSTAT, 2013).

Chickpea is a staple protein crop on the Indian sub-continent, in the Near East and across the Mediterranean basin (Ladizinsky, 1995). The protein concentration of chickpea seeds is 2 to 3 times higher than that of cereals grains. Seeds of the desi type were reported to range in protein concentration from 16.7 to 30.6%, with a mean of 23.7%, while those of the kabuli type ranged from 12.6 to 29.0%, with a mean of 20.8% (Wood and Grusak, 2007). In addition to protein, chickpea is a good source of calcium, phosphorous and some essential amino acids such as tryptophan and lysine (Jukanti et al., 2012; Hulse, 1989).

Chickpea is considered as the best dry legume for human consumption based on its high content of fiber, calcium, potassium, iron, zinc, magnesium, selenium, sodium and copper (Wood and Grusak, 2007). Thus, chickpea is a good choice of individuals with diabetes and insulin resistance because of its high fiber content (Wood and Grusak, 2007). The fiber content of chickpea is very important for lowering the blood sugar levels and preventing it from rising rapidly (McIntosh and Leu, 2001). Thus, the crop is considered as

an inexpensive source of protein and carbohydrates for the population in the developing world and a healthy choice for the world's population.

2.1.3 Chickpea production in Canada

The area sown to pulse crops in the northern Great Plains has increased steadily in the last three decades (Andrews and Hodge, 2010; Gan et al., 2010). During this period, the production of chickpea has been extended to high latitude areas such as the northern Great Plains of North America, northeast Eurasia and the Siberian steppes, northwest China, and northwest Europe (Knights et al., 2007). Expansion of area under chickpea cultivation has recently occurred in the USA, Canada and Australia due to its ability to fix atmospheric nitrogen in symbiosis with Rhizobiaceae and its economic attractiveness compared to cereal crops (Croser et al., 2003; Warkentin et al., 2003; Cutforth et al., 2007). Crop diversification was one of the main reasons for chickpea introduction to the Canadian prairies.

Several high yielding chickpea cultivars have been developed and released for production by the CDC of the University of Saskatchewan. However, most of these cultivars are late to flower and require a relatively long period to mature under the short season temperate environment. In this region chickpea maturation often occurs under unfavorable environmental conditions that are both cool and wet (Warkentin et al., 2003). Therefore, early phenology is a requirement for yield stability in this region (Warkentin et al., 2003). Time to flowering is modulated strongly by genotype, temperature and photoperiod (Went, 1953; Blázquez, 2005). The incorporation of early flowering and double podding traits has been suggested as a strategy to hasten pod filling and maturity by increasing reproductive sinks (Anbessa et al., 2007*a*). The manipulation of the genetic control of flowering time in chickpea is difficult without understanding the individual effects of genes governing this trait, interaction among them, and their response to

variation in temperature and photoperiod. Chickpea breeders have pursued research to understand the nature of genetic variation in chickpea germplasm collections for several traits and concluded that genetic diversity within the cultivated as well wild relatives could be the best sources of genes for improvement of the crop. Knowledge and management of the genetic diversity in cultivated and wild relatives are critical for any crop improvement programs. Significant variation was detected for morphological and agronomic traits in chickpea core collections. For example, exploiting cold-tolerant and vernalization responsive wild parents is a useful approach to produce cold-tolerant hybrid progeny (Abbo et al., 2003; Berger et al., 2005).

The recent advancements in genomics technologies, such as genome-wide molecular markers, genetic maps and integrative QTL analysis, facilitated the transfer of favorable alleles into elite germplasm. Introgression of single or a few genes into an adapted background can be achieved through backcrossing, pedigree systems of selection and effective phenotyping (Lander and Schork, 1994; Hittalmani et al., 2002). Marker-assisted backcrossing was applied to introgress ascochyta blight resistance and double podding traits in chickpea using molecular markers associated with these traits (Tar'an et al., 2013). Wild species could be exploited as alternative sources of resistance to biotic and abiotic stresses in varietal development in breeding programs (Gaur et al., 2012a).

2.2 Genetic diversity in chickpea

Bottlenecks in chickpea production have been attributed to several factors (Abbo et al., 2003). The same authors indicated that these factors included the limited spread of the wild relatives of chickpea to the southeastern region of Turkey unlike other Neolithic crops which have more areas of distribution of wild relatives, the founder effect associated with domestication of wild progenitors into Neolithic crops, the change from an autumn-to-spring-sowing during the early Bronze age in response to ascochyta blight, and the

replacement of landraces by elite cultivars produced by modern plant breeding. Recently, studies on polymorphism of molecular markers in chickpea indicated the presence of limited genetic variability in the cultivated species (Sefera et al., 2011). Keneni et al. (2012) on the other hand studied genetic diversity and population structure of 155 chickpea germplasm accessions, of which 139 were Ethiopian germplasm accessions. Of those, eight were released by the breeding program of Ethiopia together with eight breeding lines collected from ICARDA and ICRISAT. The authors also reported the existence of genetic diversity in these chickpea collections using 33 SSR markers. The genetic diversity reported is very limited when compared to other crops such as wheat, barley and pea which had relatively profuse genetic variation as a result of their different evolutionary histories (Upadhyaya et al., 2008).

Genetic resources enable plant breeders to create novel plant gene combinations and select crop varieties more suited to the needs of diverse agricultural systems (Brown, 1989; Glaszmann et al., 2010). Thus, exploiting the diversity in the landraces, exotics and wild relatives of chickpea in breeding programs can help raise the yield levels and enhance stress resistance level of agronomically superior cultivars (Upadhyaya et al., 2011a). Large chickpea germplasm collections are maintained by ICRISAT and ICARDA. ICRISAT alone conserves the largest chickpea collection of 20,140 accessions (19,726 cultivated, 224 wild and190 were of unknown status) and ICARDA holds a total of 13,818 chickpea collections (11,988 of the cultivated, 270 wild and 1,560 of them were of unknown status) (Upadhyaya et al., 2011b). Despite such a striking numbers of accessions, there has been only limited use of gene bank materials for the genetic enhancement of chickpea.

Brown (1989) emphasized the value and use of core collections in the utilization of germplasm collections. A core collection of chickpea (1956 accessions) well developed to represent a sub-set of the entire collection (16,991). Accordingly, a core collection of

chickpea representing about 10% of the entire collection, is a subset of accessions from the entire collection that captures most of available genetic diversity of the species. Mini-core (211 entries) are subsets of core collections, which represent 10.8% of the 1,956 core subset entries or 1.24% of entire collection. Thus, the core collections and mini-core collections were developed in such an approach that the morphological and agronomic variation contained in the entire collection of 16,991 accessions had been preserved in the core and mini core subset of 1956 and 211 entries respectively (Upadhyaya and Ortiz, 2001; Upadhyaya et al., 2002).

Exploitation of mini-core collections has provided wider prospects to the breeders for efficient utilization and economic multi-environment evaluation to identify new sources of variation for different traits for crop improvement (Upadhyaya et al., 2010). Thus, in order to facilitate the utilization of these chickpea germplasm collections in a breeding program, chickpea core subsets were developed using quantitative traits and geographic distribution of the germplasm. The traits used for the development of the core subset collection included days to 50% flowering, plant height, plant width, days to maturity, basal primary branches, apical primary branches, basal secondary branches, apical secondary branches, tertiary branches, pods per plant, seeds per pod, seed yield, 100 seed weight (Upadhyaya et al., 2011b).

A global composite collection of 3000 accessions was developed using 1956 accessions of the ICRISAT core collection, 709 ICARDA cultivated gene bank accessions, 39 advanced breeding lines and released chickpea cultivars, and 20 wild species (*C. echinospermum* and *C. reticulatum*) based on 35 distinct morphological variants. These accessions carry several traits including resistance to biotic and abiotic stress, important agronomic characters (early maturity, multi-seeded pods, double podded, large-seed size,

high seed protein, nodulation and responsiveness to high-input conditions, (Upadhyaya et al., 2006).

2.3 Biotic and abiotic stresses in chickpea production

A number of biotic constraints account for the generally low yield of chickpea globally. These include ascochyta blight, fusarium wilt, root-rots, and viruses. Chickpea is also attacked by insects including pod borer, leaf miner, storage pests and nematodes (cyst, root-knot, and lesion) (Singh et al., 1990). Abiotic stresses, such as cold, heat, drought, and salinity can cause significant yield loses (Singh et al., 1990). Cold stress during the vegetative growth stage leads to reduced yield as a consequence of reduced flow of photo-assimilates from source to sink (Thakur et al., 2010). Heat stress reduces the potential grain yield by up to 85% (Upadhyaya et al., 2010). High temperature reduced seed yield in chickpea as a result of reduced pollen production per flower, pollen viability, pollen germination and pod set was reduced by high temperature (Devasirvatham et al., 2013). High temperature reduced seed yield more than biomass in chickpea indicating that reproductive development is highly sensitive to heat. Heat tolerant chickpea germplasm lines were identified and can be utilized in breeding programs (Upadhyaya et al., 2010).

2.3.1 Ascochyta blight

Ascochyta blight is the most destructive disease affecting chickpea in many regions of the world (Singh and Reddy, 1996; Nene et al., 2012). The disease can attack at any growth stage and can affect all aerial parts of the plant, producing lesions with concentric rings of pycnidia and stem breakage due to girdling. In Saskatchewan, where the majority of Canadian chickpeas are grown, yield loss caused by an ascochyta blight epidemic was up to 96% in 1999, which was attributed to cool, wet weather, and changes in the causal pathogen population (Chongo and Gossen, 2001; Vail and Banniza, 2008). Information about the genetic bases of both virulence in the causal fungus, *Asochyta*

rabiei, and resistance in the host is essential in order to develop cultivars with more durable resistance. Durable resistance may only be possible if an array of resistance genes is combined to provide resistance against the pathogen in a single cultivar (Vir et al., 1975; Gan et al., 2006a; Anbessa et al., 2009). Relying exclusively on the resistance of current cultivars is unlikely to provide adequate disease control when conditions are favorable for disease development. The differential reaction of many chickpea lines tested in different countries indicated a considerable variability in the *A. rabiei* pathogen (Nasir et al., 2000).

Foliar fungicide application at the flowering and podding stages reduced the damage caused by the disease, and increased seed yield and quality (Chongo et al., 2003; Banniza et al., 2011). However, the environmental and economic costs of fungicides warrant evaluation of other approaches to disease control. Hence, disease resistant varieties or plant products exerting an inhibitory effect on fungal growth are being developed (Gan et al., 2007). To manage ascochyta blight in chickpea, growers are encouraged to adopt an integrated package including resistant cultivars, 4-year crop rotation, disease-free seed, and, if necessary, fungicide applications (Chongo and Gossen, 2001).

2.3.2 Drought

Drought and high temperature stresses are the major abiotic constraints of chickpea production in the Semi-Arid Tropics (SAT) and Mediterranean environments, especially in West Asia and North Africa (WANA), because in these regions the growing season is often restricted by receding soil moisture (Silim and Saxena, 1993; Singh et al., 2014). The major chickpea growing countries fall into the arid and semi-arid zones where the crop is largely grown under rainfed growing conditions. In these regions terminal drought stress is a major cause for yield losses. Yield loss as a result of drought can be prevented through crop improvement to develop better drought-adapted genotypes in food legumes (Subbarao et al., 1995). There has been a need to incorporate drought tolerance

into high yielding cultivars. In the past, breeding for resistance to drought and high temperature stresses in chickpea has been limited by the lack of adequate selection criteria (Saxena, 1990). However, because of the complex nature of drought, extensive research efforts have been made to reduce the yield loss of chickpea under drought environments (Kashiwagi et al., 2005).

In Ethiopia, chickpea is typically grown on vertisol soil in rotation with cereals including wheat, barley, and tef using progressively declining residual soil moisture at the end of the rainy season in early to mid-August (Bejiga, 1972; Anbessa and Bejiga, 2002). Chickpea is typically sown in early-October in central and south India and mid-November in Nepal. In these regions, early sowing is being practiced in order to reduce exposure of the crop to terminal drought. In these regions, cultivation of chickpea on residual soil moisture for many centuries might have shaped the crop towards better adaptation to drought stress (Saxena, 1990; Kashiwagi et al., 2005; 2006). Under terminal drought conditions, early flowering and early maturing genotypes are desired for higher yield because of their ability to escape the increasingly stressful conditions (Kashiwagi et al., 2005; Turner, et al., 2001).

Chickpea landraces which evolved in Mediterranean environments have acquired adaptation mechanisms to the drier growing conditions by increasing their root length density to capture more water than the germplasm which originated from the south Asian region (Kashiwagi et al., 2005; 2006). Desi chickpea varieties were less affected than kabuli to water stress at the seed filling phase with less damage to seed yield and accumulation of seed reserves (Nayyar et al., 2006). Genetic variability in chickpea germplasm for root traits provides opportunity for further progress on the selection and breeding for drought avoidance. Of the variation in chickpea production caused by biotic

and abiotic stress factors, nearly 50% of the variation was accounted for by water deficit (Saxena, 1990).

Early phenology (early flowering, early podding and early maturity) is the most important mechanism to escape terminal drought stress (Gaur et al., 2008). Early maturing varieties are typically not high yielding in favorable seasons due to slow total plant biomass accumulation compared to the relatively longer maturing varieties. Thus, in order to realize high yield in short growing environments, there is a need to match the crop duration with the available growing season (Gaur et al., 2008).

2.3.3 Low soil fertility

Because of low economic interest, chickpea is often grown on poor soil conditions (Kumar and Abbo, 2001).Chickpea is grown on a wide range of soils varying in texture from sand dunes in Pakistan and western Rajasthan in India, to deep vertisol clays in peninsular India, west Asia and the Ethiopian highlands. In general, the chickpea growing regions are characterized by soils with low carbon content which is an indication of low soil fertility (Ahlawat et al., 2007). Farmers in the developing countries allocate less fertile land to chickpea and the most fertile land to cereal crops, hence breeding efforts in the past focused on developing chickpea varieties adapted to infertile lands (Singh et al., 1990). On the other hand, significant improvements have been made in cereal crops in breeding cultivars responsive to fertilizer and irrigation. Poor soil and low-input conditions are the major yield limiting factors for chickpea production in most parts of the world. Exposure of the crop to adverse soil conditions affected by salinity is another cause of poor plant stands and stunted growth. In cool temperate chickpea growing regions of western Canada, soil fertility results in continuous vegetative growth (Gan et al., 2009). The continuous vegetative growth exposes the crop to ascochyta blight disease which leads to seed yield losses (Nene et al., 2012).

2.4 Photoperiodism

Photoperiodism in plants is the flowering responses to changes in the duration of day and night (Leopold, 1951). An effect of photoperiod on days to flowering of *Nicotiana tabacum* and *Glycine max* was first observed by Garner and Allard (1920). Afterwards, photoperiodism was defined as the response to the length of the day that enable living organisms to adapt to seasonal changes in their environments, with a more precise definition for plants indicating that the response is related to the timing of light and darkness (Vince-Prue, 1975, 1983). Plants are categorised photoperiodically as day-neutral (photoperiod insensitive) or sensitive to photoperiod. The day-neutral plants are those which do not respond to changes in day length. The photoperiod sensitive plants are further divided into long-day or short-day (Garner, 1933). Critical day-length was defined as the mean day length required for inducing floral initiation under natural conditions.

In short-day species, critical photoperiod is that photoperiod which, if exceeded, delays flowering or flowering would occur in a constant number of days (Major 1980). In quantitative short-day plants, there is also a ceiling photoperiod above which there is no further delay in flowering (Hadley et al, 1984). For long day plants, the critical photoperiod is that photoperiod below which there is a delay in flowering, and the ceiling photoperiod is below which there is no further delay.

In long-day plants the rate of progress towards flowering is linearly proportional to increasing day length between a ceiling photoperiod and a critical photoperiod. In short-day plants such as rice, the rate of progress to flowering increases linearly to decreasing day-length between the maximum and ceiling photoperiod and minimum critical photoperiod (Nelson et al., 2010). In general, plants are further classified as having either qualitative (obligate) or quantitative (facultative) responses based on their flowering responses under either long or short-day photoperiod regimes. Those plants in which

flowering can only occur in the inducing photoperiod have obligate response, whereas those plants in which flowering is promoted by long or short days, but which can still flower in the other photoperiods have facultative responses (Thomas and Vince-Prue, 1997). The quantitative long-day plants are those in which flowering is promoted by long-days but which can still flower under short-days as well (Vergara and Chang, 1985; Vince-Prue, 1995; Sonsteby and Heide, 2007).

2.4.1 Photoperiod sensitivity

Photoperiod sensitivity is the derivative of the function relating time to flowering to day-length in non-optimal photoperiod conditions (Vergara and Chang, 1985). Photoperiod sensitivity is expressed in units of days delay per hour increase or decrease of day length (Major, 1980). In many cereal crops, response to flowering was used as an indirect measure of photoperiod sensitivity (Yano et al., 2001). In chickpea, time to flowering was used as a direct measure of photoperiod sensitivity (Roberts et al., 1985; Anbessa et al., 2006).

The difference in photoperiod sensitivity is important in the adaptation of a particular crop species to production areas in different latitudes and seasons (Bashandi and Poehlman, 1974). This was true as a result of selection for survival which adjusted flowering and maturity to the most productive time relative to the availability of soil moisture. In other words photoperiod sensitivity limits geographic adaptation of plants by affecting days to flowering through genetic control and interaction with other flowering genes (Murfet, 1977). For example, identification of flowering response of rice to photoperiod contributed to the wide geographic distribution of the crop (Izawa, 2007). Similarly, the productivity of soybean [(*Glycine max* (L.) Merr.)] in the short day photoperiod, warm tropics occurred as a result of selection for a relatively long duration of the photoperiod-insensitive pre-inductive phase in the crop (Collinson et al., 1993).

Likewise, selection for day-neutral genotypes played a great role in wider adaptation in wheat and rice (Worland et al., 1998). In most photoperiod insensitive cereals, initiation of floral primordia takes place without the requirement for long-day photoperiods, whereas photoperiod sensitive genotypes need a long-day photoperiod for floral primordial initiation to take place (Stracke and Borner, 1998).

Photoperiod insensitivity played an important role in chickpea adaptation to low latitude environments during early chickpea domestication. In the Mediterranean basin, photoperiod insensitivity was associated with early flowering in order to escape moisture stress, which occurs frequently during the summer months. Thus, early flowering is positively associated with grain yield in the Mediterranean environments (Siddique et al., 2003; Rubio et al., 2004).

2.4.2 The genetic control of photoperiod sensitivity

Understanding the duration when photoperiodic plants become sensitive to inductive photoperiods is decisive for appropriate crop management, allowing growers to either advance early flowering to reduce crop duration or purposely delay flowering (Warner, 2009). The photo-thermal response of plants during their pre-flowering developmental phase plays an important role in matching crop duration to the favorable production environment for the best use of the growing season and to avoid loss of yield and quality as a result of adverse climatic factors (Cutforth et al., 2007).

Several studies have investigated the effects of photoperiod at different times during pre-flowering development of crops including rice (*Oryza sativa* L.) (Collinson et al., 1992). In rice, photoperiod along with temperature is the most important environmental factor that determines the timing of flowering and seed setting. Maize is sensitive to photoperiod at the stage of tassel initiation (Kiniry et al., 1983). Wang et al. (2008) reported a significant reduction of time to flowering, increased number of leaves and plant

height under long days as compared to short-days in maize recombinant inbred lines derived from a cross between parents of temperate and tropical origin. Soybean accessions which originated from higher latitude countries were reported to be day-neutral. In these accessions, photoperiod influenced the total days of early growth and floral bud development (Shanmugasundaram and Tsou, 1978; Wilkerson et al., 1989; Collinson et al., 1993). In lentil and chickpea the effects of wide ranges of photo-thermal conditions on rates of progress towards flowering was reported as a linear function of temperature (Roberts et al., 1985; Summerfield and Roberts, 1988).

In most plants, similar to time to flowering, the genetics of photoperiod sensitivity is very complex. QTLs identified for days to flowering and photoperiod sensitivity in cereals such as barley, oat, maize, sorghum, rice and wheat have indicated the presence of orthologous genes in these species (Lin et al., 1995). In barley, photoperiod determining genes were identified on five of the seven chromosomes (Stracke and Borner, 1998). In wheat photoperiod insensitivity is primarily determined by a homoelogous series of dominant genes located on the group 2 chromosomes (Worland, 1996). In pea, photoperiod response mutants exist in two forms, i.e., early day-neutral and late day-neutral. The early day-neutral mutants perform under short-day conditions as if grown under long-days whereas the late day-neutral mutants perform under long-day as if grown under short-days. These photoperiod response mutants affect the circadian clock or light perception in addition to all photoperiod aspects of growth such as flower induction (Weller et al., 2009).

Chickpea is inherently a long-day plant (van der Maesen, 1972; Summerfield et al., 1981). Several day-length insensitive lines have been identified originating from low-latitude areas in East Africa, India, Mexico and Iran. Another extra-early flowering cultivar ICCV 96029 developed by ICRISAT is being grown in the low land area of India where

terminal moisture stress is a major production constraint. It was derived from a cross between ICCV 2 and ICCV 93929 (Kumar and Rao, 2001). ICCV96029 is being used as a parent to develop early flowering lines for the short-duration environments of western Canada (Kumar and Abbo, 2001; Anbessa et al., 2006). ICCV 2 was the first breakthrough kabuli variety in short-season environments (Kumar et al., 1985). In chickpea there is limited information about the mechanism of photoperiod sensitivity and duration of the photoperiod sensitive phase.

There is a high morphological variability in chickpea; but low genetic diversity. The low genetic diversity in chickpea could be due to monophyletic descendence from its wild progenitor *C. reticulatum* (Keneni et al. 2012; Abbo et al., 2003; Ladizinsky and Adler, 1976). The genetic diversity in the cultivated and wild germplasm collections could be exploited to produce better genetic recombination and segregation in their progenies resulting in varieties with broad genetic base (Chahal and Gosal, 2002). For some traits such as vernalization response it might be necessary to use wild ancestors of crop plants to introgress some of the diversity that was lost during the domestication process in order to improve agricultural yields under optimal as well as stress conditions (Varshney et al., 2005).

2.5 Flowering time in chickpea

2.5.1 Physiological basis of flowering in chickpea

In many crops, a minimum vegetative period, known as the basic vegetative phase, is required during which there is no response to photoperiod (Vergara and Chang, 1985). In cereals, the duration to flower consists of three developmental phases: an initial pre-inductive photoperiod-insensitive (juvenile) phase, a later photoperiod-sensitive inductive phase and a reproductive phase (panicle initiation to flowering) (Major, 1980; Vergara and Chang, 1985). Timing of floral induction is determined by the juvenile phase,

a plant development phase between germination and when a plant is sensitive to photoperiod. However, in the later photoperiod sensitive phase plants can change from vegetative to reproductive if exposed to an inductive photoperiod (Thomas and Vince-Prue, 1996). If plants are exposed to non-inductive photoperiod immediately after receiving the inductive condition, reproductive primordia can revert back to vegetative condition, producing leafy structures instead of a panicle (Ong and Everard, 1979)

Knowledge of the photo-thermal effect on time to flowering in plants is essential because flowering stage is the key factor in plant adaptation to variable environments. In chickpea, physiological studies indicated that temperature and photoperiod are major determinants of time to flowering (Roberts et al., 1985). The vegetative growth phase under either short or long photoperiods had a negative relationship with the mean diurnal temperature (Roberts et al., 1985). In sorghum, a short day plant, selection for germplasm with a long juvenile phase facilitated the development of early sorghum varieties that have agronomic advantages and are adapted to regions near to the equator (Alagarswamy et al., 1998). Similarly, utilization of cultivars with a longer juvenile phase has been reported for successful low-latitude adaptation of soybean which had originated from higher latitudes (Ray et al., 1995).

The two major genes reported in chickpea by Anbessa et al. (2006) might determine the flowering response to temperature and photoperiod. Berry and Aitken (1979) reported that in the earliest flowering pea varieties, days from sowing to first flower were inversely proportional to temperature with no effect of photoperiod. The same authors reported that in intermediate and highly photoperiod sensitive accessions, flowering response to photoperiod was temperature-dependent.

2.5.2 Genetic basis of flowering time in chickpea

Flowering is a complex process regulated by the interaction of many genes within an organism and also influenced by environmental factors (Murfet, 1977). Genes which control the time of flowering are divided into vernalization response genes, photoperiod response genes, and earliness *per sensu stricto* genes. The earliness *per ss* genes function independent of environmental effects (Coupland, 1995). In chickpea, time to flowering is determined by two major environmental factors: photoperiod and temperature (Roberts et al. 1985). Summerfield et al., (1988) reported that there was no specific vernalization response in cultivated chickpea genotypes, whereas more recent reports indicated the existence of vernalization responses in wild chickpea accessions (Berger et al., 2005).

Three major environmental variables, photoperiod, vernalization, and ambient growth temperature affect flowering time in *Arabidopsis* (Lempe et al., 2005). In addition to the 80 genes contributing to floral transition, several environmental and endogenous pathways also play significant roles in controlling flowering time in *Arabidopsis* (Simpson and Dean, 2002).

In chickpea, a major flowering gene (PPD) was identified based on a 3:1 segregation of late: early individuals among the F_2 progeny of a cross between the relatively late-flowering Hadas and the early flowering ICC 5810 cultivars (Or et al., 1999). A required temperature range of 14 to 26 ˚C for flowering was reported in the early flowering genotypes (ppd/ppd). In these genotypes, the photoperiod requirement was very low to initiate flowering resulting in early flowering in seasons characterized by sufficient temperatures combined with short photoperiods. On the other hand, in photoperiod-sensitive types (PPD/–), flowering is delayed until photoperiod requirements are satisfied (Hovav et al., 2003). Chickpea genotypes originating from the tropics have been grown for

millennia in environments where seasonal day-length variation is minimal, and as a result they have acquired a lower degree of photoperiod sensitivity than those of Mediterranean origins (Or et al., 1999).

Two complementary major genes were reported to determine time to flowering in chickpea in the high latitude, cool-season environments of western Canada and these genes determined 65% of the phenotypic variation with minimal contribution of the polygenes (Anbessa et al., 2006). There is limited information about the reaction of these two major genes with photoperiod and temperature. Information acquired about the interaction of these genes with the environmental factors would enable further understanding and manipulation of the genetic system required for the development of chickpea varieties matching the growing condition of western Canada. Several authors have reported genes governing flowering time in chickpea (Table 1.1). The differences in the type and number of genes reported by these authors could be attributed to the differences in genetic background of the test materials used and, most significantly, to differences in environmental conditions such as photoperiod and temperature.

2.6 Molecular breeding in chickpea

2.6.1 Molecular breeding for time to flowering in chickpea

Flowering is a complex trait which is the end result of numerous physiological and biochemical processes within a plant. Flowering time genes interact with environmental factors to regulate the flowering processes in plant species (Murfet, 1977). Flowering time genes are important for crop adaptation to a particular environment (Worland, 1996). The same author indicated that there is a highly significant association of flowering time genes and plant height, biomass, and yield components in wheat.

Table 1.1. Summary of important genes and QTLs associated with flowering time and photoperiod response in chickpea

Number of genes/mode of inheritance	Population	References
One major gene: *PPD/ppd* (dominant/recessive)	Hadas and ICC 5810	Or et al., 1999
Efl1/ efl1 (dominant/recessive)	F_6 RILs derived from cross between ICCV-2 X JG-62	Kumar and Rheenen, 2000
Two major genes and polygenes	$F_{2:3}$ derived from crosses between 272-2 X CDC Anna, 298T-9 x CDC Anna, and 298T-9 x CDC Frontier	Anbessa et al., 2006
Duplicate dominant genes	Dominant alleles (*Efl1*, *Efl2*) - late flowering	Hegde, 2010
	Dominant alleles (*Efl1*,*efl2*) - early flowering	
	Homozygous recessive alleles (*efl1*, *efl2*) - extra-early flowering	
Dominant gene (*Efl3*)	BGD 132	Hegde, 2010
Efl4	(ICC 16641/ ICC 16644)	Gaur et al., 2014
QTL (2) on LGs 1 and 8	Crosses between Hadas X ICC 5810	Lichtenzveig et al., 2006
QTL (2) on LG3	F_{10} derived RILs derived from ICCV-2 X JG-62	Cho et al., 2002
QTL (1) on LG4	$F_{6:7}$ RILs derived from a cross between CA2156 X JG-62	Cobos et al., 2007
QTL(1) on LG3	$F_{6:7}$ RILs derived from a cross between ICCL81001 (*Cicer arietinum)* and Cr5-9 (*Cicer reticulatum)*	Cobos et al., 2009
QTL (2) on LG3	F_2 derived from a cross between ICC 3996 (*Cicer arietinum)* X ILWC 184 (*Cicer reticulatum)*	Aryamanesh et al., 2010
QTL (1) on LG8	Hadas	Zhang et al., 2010
QTL (4) on LGs,1,3,4, and 8	RILs derived from a cross between ILC 588 XILC 3279	Rehman et al., 2011
QTL (1) on LG3	$F_{2:3}$ derived from a cross between ILC 3279 X ICCV2.	Jamalabadi et al., 2013

Understanding of the effect of these genes is crucial to maximize plant performance. Information on the genetics of time to flowering in chickpea genotypes facilitates breeding of chickpea varieties to target environments. Anbessa et al. (2006) reported two major genes and polygenes to affect flowering time in chickpea in the short season of western Canada. Or et al. (1999) reported the presence of major genes in chickpea genotypes. These different reports on the inheritance of flowering time genes were attributed to differences in the accessions used by individual researcher and also environments where the experiments were conducted.

Several studies reported genetic inheritance of flowering time in chickpea recombinant inbred line population. Jamalabadi et al. (2013) reported a QTL for days to flowering on linkage group 3 using RILs derived from ILC 3279 and ICCV 2. Genetic mapping for time to flowering using chickpea recombinant inbred lines derived from a cross between the Israeli cultivar Hadas and the Indian accession ICC5810 revealed two QTLs for flowering time, one on LG1 and another on LG2 (Lichtenzveig et al. 2006). Similarly, Cobos et al. (2007), reported a QTL for days to flowering in linkage group 4 using a chickpea recombinant inbred line population derived from an intraspecific cross between CA2156 and JG62. QTLs for resistance to ascochyta blight, seed size, seed coat thickness, seed yield, genes for flower colour have been located on this linkage group in addition to QTLs for flowering time (Santra et al. 2000; Millan et al. 2010; Tekeoglu et al. 2000; Collard et al. 2003; Flandez-Galvez et al. 2003; Udupa and Baum, 2003; Cho et al., 2004; Cobos et al. 2006; Iruela et al., 2007). Thus linkage group 4 is an important genomic region for most agronomic traits in chickpea.

2.6.2 Achievements in quantitative trait loci mapping in chickpea

Molecular markers linked to major QTLs contributing resistance to ascochyta blight have been discovered and could be deployed in marker-assisted breeding (Tekeoglu et al.,

2002; Flandez-Galvez et al., 2003). The use of molecular markers facilitates the pyramiding of resistance genes from diverse sources and should significantly reduce the time required to develop resistant cultivars (Santra et al., 2000; Tar'an, et al., 2013).

Genomic mapping of the chickpea genome has been of interest to identify genomic locations of important traits, particularly early flowering, maturity and ascochyta blight resistance. For example, most QTLs for ascochyta blight resistance and a high number of genes for morphological traits were anchored on LG2 and LG4 in 10 mapping populations studied by Millan et al. (2010). More focus should be given to these linkage groups in molecular breeding. Marker-assisted selection for ascochyta blight resistance would greatly accelerate the development of new chickpea cultivars (Millan et al., 2003). Identification of markers linked to traits of agronomic importance is very useful for mapping and tagging of the genes or QTL governing genes related to resistance to biotic and abiotic stresses in chickpea breeding.

A focus on variation in candidate genes or DNA markers closely linked to the identified QTLs is the most appropriate strategy (Varshney et al., 2005). QTL hotspots based isolation of candidate would facilitate a transfer of large size chromosomal intervals from a donor parent into recurrent parent (Zou et al., 2012). The co-localization of candidate genes with QTLs controlling a particular phenotype supports the use of the candidate gene as a potential source to develop perfect markers for selecting the phenotype in marker-assisted breeding (Varshney et al., 2005).

In chickpea, several genes for abiotic responses have been identified on the basis of sequence similarity between candidate genes previously validated for their significance in stress responses in various model crops and other legumes. These traits include drought stress, salt stress, wound healing, ethylene responsiveness, response to cold (Roorkiwal and Sharma, 2012).

2.6.3 Recent advances in genomic technologies and application in chickpea breeding

In recent years significant progress has been made in the development of chickpea genetic and genomic resources. Large-scale genomic resource development in chickpea since 2010 include large-scale, high-throughput EST sequencing projects (Hiremath et al., 2011, Garg et al., 2011), development of SSR and SNP markers from an SSR-enriched genomic library, BAC libraries and transcriptome sequences (Nayak et al., 2010; Hiremath et al., 2011). As a result several high-throughput SNP genotyping platforms have been developed and were successfully used for construction of high-resolution linkage maps (Thudi et al., 2011, Gaur et al., 2012b; Deokar et al., 2014) and germplasm diversity analysis (Roorkiwal et al., 2013, Diapari et al., 2014).

A new era of chickpea genomics started with the availability of whole genome sequences of multiple chickpea genotypes. The draft sequence of a desi-type chickpea (ICC 4958) genome was generated using next-generation sequencing platforms, bacterial artificial chromosome end sequences and a genetic map (454/Roche GS FLX Titanium platform) covering 520 Mb of 740 Mb containing 27, 571 genes was generated (Jain et al., 2013). The whole genome shotgun draft sequence of CDC Frontier, a kabuli chickpea variety, covered a genome size of 738 Mb and about 28,269 genes (Varshney et al., 2013). Re-sequencing information of 90 cultivated and wild chickpea genotypes also provided additional information about breeding-associated genetic sweeps and breeding-associated balancing selection (Varshney et al., 2013). A separate study using chromosomal genomics approach revealed the resemblance of the physical genomes of the two chickpea types (Ruperao et al., 2014).

Linkage maps with sequence-based molecular markers such as SNPs allows direct comparison of linkage maps with the physical map which provides a foundation for cloning and isolation of QTLs/genes for molecular dissection of traits, as well as markers for

molecular breeding (Varshney et al., 2014a). Comparisons of linkage maps with the first generation chickpea physical map identified three large contigs associated with QTLs for ascochyta blight resistance and days to flower (Zhang et al., 2010). Recently, potential candidate genes located in the QTL-hotspot (QTL for yield-related traits and drought indices) were identified using a linkage map and the CDC Frontier physical map (Varshney et al., 2014a). In another study, 306 and 23 candidate genes located in QTLs for ascochyta blight and fusarium wilt resistance, respectively, were identified (Varshney et al., 2014b).

The association of these genetic maps with phenotyping information of the respective segregating mapping population has facilitated the identification of molecular markers linked with the genes or QTLs controlling several agronomically important traits (Das and Parida, 2014). Accordingly, genetic dissection of chickpea for drought tolerance was conducted using genotyping by sequencing and identified QTL‐hotspot regions for drought tolerance (Thudi et al., 2014) and ascochyta blight resistance (Stephens et al., 2014). Identification of such regions facilitates a marker-assisted selection approach for introgression of such regions derived from parental germplasm in chickpea breeding programs. Existence of natural allelic diversity provided the insight to utilize wild chickpea as a resource for trait enhancement in the cultivated gene pool (Saxena et al., 2014). The availability of these tools aid breeders for implementing integrated efficient breeding approaches for cultivar development (Varshney et al., 2014b). Availability of the chickpea genome sequence facilitated the identification of genomic regions controlling root traits and several other traits related to drought tolerance (Gaur et al., 2012b) and micronutrient concentrations (Diapari et al., 2014). Exploiting these genomic resources may help to hasten development of chickpea cultivars adapted to the western Canadian environments. The first chapter was conducted to address flowering response of diverse chickpea accessions to photoperiod.

3. Flowering Response of Diverse Chickpea Accessions to Photoperiod

Abstract

Knowledge of the time required for the initiation of flowering is important for chickpea adaptation in environments like western Canada, not only because flowering is a vulnerable stage of development but also it is a major factor affecting variation in crop duration. Temperature and photoperiod are the major environmental factors that determine time to flowering in many major crops. One hundred diverse chickpea accessions were examined under long day (16/8 hours day and night, respectively) and short day (10/14 hours day and night, respectively) photoperiods for their flowering response in growth chambers. The temperature of the chambers was adjusted to 22/16˚C (day and night, respectively). Variability among the accessions for days to flowering, photoperiod sensitivity, and node number of first flower under short and long day photoperiods was detected. Four accessions were classified as photoperiod-insensitive, 49 as intermediate and 47 as highly photoperiod-insensitive based on their flowering response to photoperiod. A range of response from day neutral to highly sensitive to photoperiod was identified. A significant ($r = 0.92$, $P \leq 0.0001$) positive correlation was observed between days to flowering under short days and photoperiod sensitivity. In photoperiod-insensitive cessions fewer degree days were recorded as compared to intermediate and late flowering ones. Under long day photoperiod, the photo-thermal and thermal units required to initiate flowering accumulate faster. Thus photoperiod-insensitive accessions required fewer accumulated thermal units and photo-thermal units.

3.1 Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinated, diploid (2n = 2x = 16) plant and the second most widely grown grain legume crop after dry bean (*Phaseolus vulgaris* L.) (Singh, 1997; FAOSTAT, 2013). Expansion of the area under chickpea cultivation has recently occurred in the USA, Canada and Australia due to its ability to fix atmospheric nitrogen in symbiosis with *Rhizobium* (Gan et al., 2003) and its economic attractiveness compared to cereal crops (Croser et al., 2003; Cutforth et al., 2007). Chickpea is one of the legumes that fit well into rotations with cereal and oilseed crops (Zentner et al., 2002).

In western Canada, due to the short crop growing season available for chickpea (110-120 days), maturity often coincides with end-of-season frost resulting in severe losses in grain yield and quality (Warkentin et al., 2003). The time required for the initiation of flowering in environments like western Canada needs to be sufficiently long to allow the crop to accumulate enough photosynthate for adequate yield, but short enough to fit the available growing season (Levy and Dean, 1998; Warkentin et al., 2003). Early flowering and podding restrict vegetative growth in indeterminate crops like chickpea and thereby hasten time to maturity (Ladizinsky and Adler, 1976; Berger et al., 2006; Anbessa et al., 2007*b*). Temperature and photoperiod are the major environmental factors that determine the time to flowering in crops (Coupland, 1995; Roberts et al., 1993). Genetic analysis of flowering time and its bearing on agronomic performance is fundamental to crop improvement for better adaptation (Kumar and Abbo, 2001).

Chickpea has generally been considered as a quantitative (facultative) long-day plant (Van der Maesen, 1972; Sethi et al., 1981). Recent findings, however, indicate that chickpea can also be considered as a qualitative (obligate) long-day plant in which flowering does not take place at photoperiod lower than a critical value of 11 to 12 hours (Soltani et al.,

2004). In some chickpea genotypes, flowering time is influenced both by photoperiod and temperature, whereas in others it is determined solely by photoperiod (Ellis et al., 1992).

In soybean, the influence of photoperiod on flowering was studied using a wide range of genotypes and environments (Summerfield et al., 1998). There were significant effects of temperature, photoperiod, and a temperature and photoperiod interaction for days to first flower (Cober et al., 2001). A total of thirteen maturity groups ranging from MG000 to MGX were classified in North America (Cober and Voldeng, 2001). The availability of these maturity groups with high diversity in time to flowering and maturation was reported in soybean cultivars (Bingjun et al., 2014).

Availability of chickpea cultivars which are early flowering and early seed setting under short photoperiods indicates the potential for breeding the crop with wider environmental adaptability (Sandhu and Hodge, 1971). However, research into the benefits of photoperiod insensitivity is very limited, therefore there, is need for more research on the effects of photoperiod, particularly the link between sensitivity to photoperiod and flowering response for the overall adaptation of the crop to the short growing season of western Canada.

In wheat, the development of cultivars which are less sensitive to photoperiod contributed to the adaptation of the crop to a wide range of environments from the equator to 67 ˚N and 45 ˚S (Kamran et al., 2014). Photoperiod insensitivity has the strongest effect in promoting flowering in wheat before the onset of long days in areas where the plant is required to flower before the onset of warm, desiccating temperatures of spring and summer, and to the areas where it is sown during a short winter season (Snape et al., 2001). Tsubokura et al. (2013) reported the wide adaptability of soybean (*Glycine max*) from the equator to high latitudes of at least 50 ˚N was attributed to natural variation in a number of major genes controlling flowering.

Identification of genetically diverse germplasm is crucial for crop improvement programs in grain legumes (Kamran et al., 2014). Sethi et al. (1981) reported that in chickpea flowering is accelerated under long days of 15 hours combined with temperature regimes ranging from 18 to 30 ˚C. A combination of photoperiod and temperature insensitivity is required to ensure early flowering in chickpea (Or et al., 1999). Thus, the development of chickpea varieties which will flower and mature sufficiently early to avoid terminal frost stress in the Canadian prairies is an important research priority. However, information on the variability among diverse chickpea accessions is inconclusive. The main objectives of this study were to evaluate the flowering response of 100 diverse chickpea accessions grown with either long or short-days, and to examine the photoperiod sensitivity of the chickpea accessions.

3.2 Materials and Methods

3.2.1 Germplasm accessions

Kabuli and desi chickpea accessions from the gene bank at the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India and kabuli accessions from the International Center for Agricultural Research in the Dry Areas (ICARDA) (formerly at Aleppo, Syria) together with selected cultivars from the Crop Development Centre, University of Saskatchewan totaling 100 accessions were evaluated in this research (Appendix I). Seeds of these accessions were multiplied in the breeding nursery at Kyle, Saskatchewan, in summer 2009 to maintain uniformity of the seed source prior to the experiment. For simplicity, all genotypes evaluated will be referred to as 'accessions'.

3.2.2 Photoperiod treatments

The chickpea accessions were evaluated under long and short days in growth chambers. Two time replicates were conducted for each photoperiod. The first and second time replicates under long days were conducted from June-September, 2010 and November 2010-February 2011, respectively. The first and second time replicates under short days were conducted August-November, 2010 and May-August, 2011, respectively.

The accessions were grown in separate growth chambers, one with long day photoperiod (16 hours light), and the other with short day photoperiod (10 hours light). Both chambers were adjusted to 19 ºC mean temperature (16 ºC night temperature [dark period] and 22 ºC day temperature [light period]) and relative humidity of 70 ± 2.5%. The chambers were equipped with light bulbs (T12 VHO 96'' Long white; 4200 k, and 60W Incandescent Red) with a maximum capacity of 250 to 300 µmol $m^{-2}s^{-1}$. Three seeds of individual accessions were grown in a 3.8 L pots containing Sunshine mix media #4 (Sun Gro, Seba Beach, AB). Three replicate pots of each accession were grown in each chamber in a completely randomized experimental design. Plants were watered every 2 to 4 days depending

on crop growth stage. Two weeks after seeding and full emergence, the seedlings were thinned to two plants per pot. Once a week the plants were fertilized with a quick release fertilizer (20 N: 20 P_2O_5:20 K_2O) at 3 g/l beginning one week after emergence.

3.3 Data collection and analyses

Days to flowering was recorded as the number of days from seedling emergence to first flower opening (corolla color visible) on individual plants of each accession under each photoperiod treatment. The number of days to flowering under long-days was subtracted from the number of days to flowering under short-days for each accession. The difference in days to flowering under short days as compared to long days is considered as the photoperiod sensitivity. Node number of first flower was measured by counting the number of nodes on the main stem and/or along the branch carrying the first open flower following a similar procedure used by Roberts et al. (1985).

The average score from two plants in a pot were used for data analyses. Data were analyzed using PROC GLM of SAS 9.3 (SAS Institute Inc., Cary, NC). Analysis was initially conducted for each experimental time replicate under long and short day photoperiods, followed by a combined analysis. Homogeneity of variance was assumed due to the controlled environmental conditions used. In some of the accessions, seedling emergence was not uniform across the three replications leading to some missing data points and different treatment degrees of freedom in time replicates 1 and 2 (Table 2). Pearson correlation coefficients for days to flowering, node number of first flowering and photoperiod sensitivity were calculated across the accessions averaged over two time replicates using PRO CORR of SAS 9.3 (SAS Institute Inc., Cary, NC).

The thermal unit (heat unit) is the summation of the mean daily maximum and minimum air temperature in the growth chambers as described by (Summerfield and Roberts 1988, McMaster and Wilhelm 1997). A photo-thermal unit is the product of mean daily

temperature and duration of light time under long day photoperiods. These two units were calculated for the photoperiod conditions using a formula: Considering 16 H for the long day photoperiod and 10 H for short day photoperiod and T_{max} = 22 ºC, and T_{min} = 16 ºC for day and night temperature respectively.

Thermal units = (Day H x T_{max}) + (Night H x T_{min})/24 H

Thermal units under long days = (16 H x 22 ºC) + (8 H x 16 ºC)/24 H

Thermal units under short days = (10 H x 22 ºC) + (14 H x 16 ºC)/24 H

Where: T_{max} and T_{min} are maximum and minimum daily air temperatures, respectively.

Photo-thermal units = (day H x day temperature)/24 H

Photo-thermal units under the long days = (16 H x 22 ºC)/24 H

Photo-thermal units under the short days = (10 H x 22 ºC)/24 H

The summation (cumulative) thermal units and photo-thermal units between seedling emergence and first flower appearance under long and short days were calculated as the product of number of days to flowering and the daily accumulated thermal and photo-thermal units respectively. Correlation coefficients between days to flowering and cumulative thermal and photo-thermal units under long and short days were detected. Similarly, regression analysis was conducted for the number of days to flowering against the total thermal units and photo-thermal units between seedling emergence and first flower appearance under long and short days.

3.4 Results

3.4.1 Days to flowering and node number of first flower

In some of the accessions, seedling emergence was not uniform across the three replications leading to some missing data points and different treatment degrees of freedom in time replicates 1 and 2 (Table 3.1). Due to seed shortage, measurements were not taken on different days for separate replicate pots. Highly significant ($P \leq 0.001$) differences were observed among the chickpea accessions for their days to flowering, node of first flower, and photoperiod sensitivity under long and short days. The mean number of days to flowering was 35 days under long days and 68 days under short days. The earliest accessions flowered in 24 days under long days and in 28 days under short days (Figure 3.1). The latest flowering accessions flowered in 50 days under long day and 105 days under short day photoperiods, respectively.

Significant differences ($P \leq 0.05$) in the number of days flowering and node number of the first flowering on the chickpea accessions under long and short days in both time replicates were detected. The average node number to first flowering was 14 nodes under long-day photoperiod and on 22 nodes under short-day photoperiod.

Based on the significant differences detected among the chickpea accessions in their flowering responses under long and short-days, the accessions were categorized into different photoperiod sensitivity groups ranging from 5 days photoperiod sensitive period in the day-neutral, to 65 days in photoperiod sensitive accessions (Figure 3.2). Accessions with 1 to 10 days difference in the number of days to flowering under short days compared to long days were grouped as photoperiod-insensitive (day-neutral). Accessions with 10 to 40 days difference in the number of days to flowering under short-days versus long-days were considered as intermediate in their photoperiod-sensitivity. Accessions whose days to

flowering were delayed for 41 days or longer were in the category of highly sensitive to photoperiod.

Table 3.1. Long and short-day photoperiod treatment effects on the number of days to flowering, number of node to first flower and photoperiod sensitivity of diverse chickpea accessions evaluated in growth chambers in 2010-2011.

Photoperiod	Time replicate	Characters	df			F-value
			Treatment	Error	Total	
Short days	1	Days to flowering	96	163	259	4.3***
		Node of first flower	96	135	231	25.7***
	2	Days to flowering	85	111	196	6.1***
		Node of first flower	85	134	219	4.2***
	Combined	Days to flowering	99	261	360	6.3***
		Node of first flower	99	156	255	3.6***
Long days	1	Days to flowering	84	134	218	2.8***
		Node of first flower	84	103	187	3.9***
	2	Days to flowering	97	182	279	6.2***
		Node of first flower	97	159	256	4.5***
	Combined	Days to flowering	98	249	347	5.6***
		Node of first flowering	98	231	329	2.3***
	1	Photoperiod sensitivity	94	150	244	2.9***
	2		81	120	201	5.9***
	Combined		97	249	346	5.3***

Note: df = degree of freedom; *** indicates significant difference at $P \leq 0.001$.

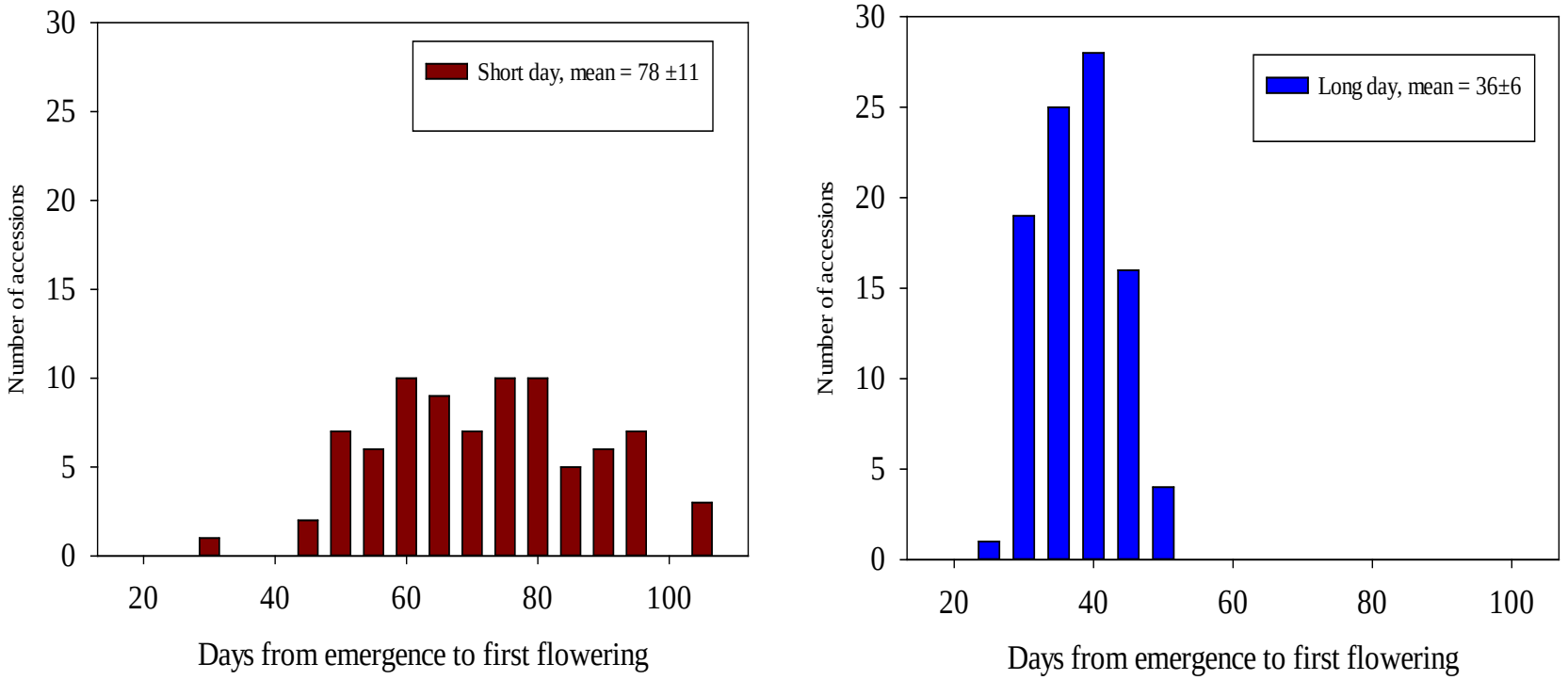

Fig. 3.1 Distribution of 100 chickpea accessions based on days to flowering evaluated under long-day and short-days. Data were averaged from two time replicates.

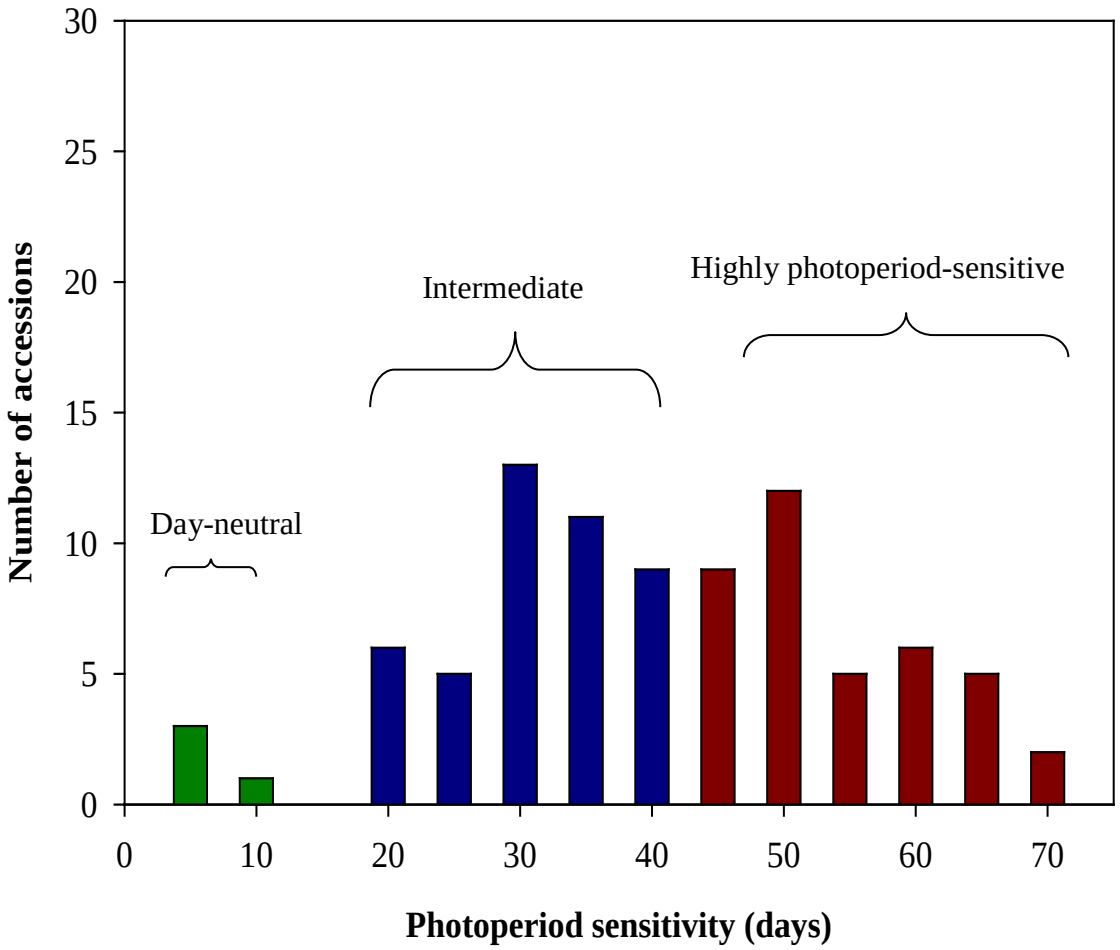

Fig. 3.2 Distribution of the 100 chickpea accessions based on their degree of photoperiod sensitivity (day-neutral, intermediate and highly photoperiod sensitive). The accessions were evaluated in growth chamber under long and short-days. Photoperiod-sensitivity was calculated as a delayed number of days to flowering of the accession under short-day compared to long-day photoperiod

3.4.2 Correlation between days to flowering and number of node of first flower

Correlations among days to flowering, node number of first flower and photoperiod sensitivity were estimated. The resuts showed that the magnitude of the correlations were significantly different under the different photoperiods (Table 3.2). Significant and positive correlation between long-day and short-day conditions for days to flowering ($r = 0.22$, $P \leq 0.0001$) and node number of first flowering ($r = 0.22$, $P \leq 0.0001$) were detected. The highest correlation coefficients were detected between photoperiod sensitivity and days to flowering ($r = 0.92$, $P \leq 0.0001$), and between photoperiod sensitivity and node number of first flowering ($r = 0.62$, $P \leq 0.0001$) under short-day conditions. Under long-day conditions, although the correlations were relatively weak, photoperiod sensitivity was significantly correlated with the number of days to flowering ($r = 0.13$, $P \leq 0.01$), and node number of first flowering ($r = 0.11$, $P \leq 0.02$). Under short-day conditions, significant and positive correlations were observed between days to flowering and node number of first flowering ($r = 0.70$, $P \leq 0.0001$). However, number of days to flowering was not correlated with node number of first flowering under long-day conditions.

Table 3.2: Pearson correlation coefficient for days to flowering and node number of first flowering under long and short-day photoperiod conditions and photoperiod sensitivity (averaged over two experimental time replicate) of 100 chickpea accessions.

Characters	Dtf_LD (31 ± 7)	Node_SD	Node_LD	Dtf_SD
NodeSD (22 ± 5)	0.22^{***}			
NodeLD (14 ± 4)	0.01^{ns}	0.10^{ns}		
DtfSD (70 ± 19)	0.22^{***}	0.70^{***}	0.25^{***}	
PS (38 ± 17)	0.13^{**}	0.62^{***}	0.11^{*}	0.92^{***}

Note: Dtf_LD = days to flowering under long-days,
Dtf_SD = days to flowering under short-days,
Node_SD = node number of first flowering under short-days,
Node_LD = node number of first flowering under long-days,
PS = Photoperiod sensitivity defined as the delayed number of days to flowering under short-day photoperiod treatment as compared to long-day photoperiod treatments.

Numbers in the brackets represent the mean values for the characters were averaged over three replications in two time replicates for each accession.

*, **, *** indicates significant correlation at $P \leq 0.05, 0.01, 0.001$, respectively, and
ns = non-significant correlation. Photoperiod-sensitivity was counted as delayed in days to flowering of the accession under short day as compared to long-day photoperiod.

3.4.3 Photoperiod sensitivity

Photoperiod sensitivity was calculated as the difference in the number of days to flowering under short days and long days for each chickpea accession. Photoperiod sensitivity was calculated as the difference in the number of days to flowering under short days and long days for each chickpea accession. A wide range of photoperiod sensitivity was observed. Four day-neutral chickpea accessions were identified, i.e. ICCV 96029 and ICC 8350 (desi), ICC 12968 and FLIP 98-142C (kabuli). For these accessions the number of days to flowering under short days was delayed by 1 to 10 days compared to under long days (Appendix II and Figure 3.2). The 49 accessions which flowered 10 to 40 days later under short days as compared to long days were categorised as intermediate sensitivity to photoperiod. This group was comprised of 25 kabuli accessions and 24 desi accessions. A total of 47 accessions whose days to flowering was delayed by 41 to 65 were categorised as highly sensitive to photoperiod. This category consisted of 14 desi and 32 kabuli accessions.

3.4.4 Effect of thermal units and photo-thermal units on days to flower

The thermal time concept was applied in photoperiod-insensitive genotypes based on the observation that progress toward flowering is a linear function of temperature (Summerfield and Roberts 1988). Thermal time calculations usually involve three cardinal temperatures (i) a base temperature (T_b) below which no development occurs, (ii) an optimum temperature (T_o) at which development proceeds at a maximum rate and (iii) a maximum temperature (Tm) above which no development occurs.

The derived values of thermal units and photo-thermal units were used as a quantitative index of the temperature and photoperiod regimes. The derived value of the thermal units under long days was 20 degree-days and the value under short days was 18.5 degree days. Similarly, the derived value of the photo-thermal unit under long days was

calculated to equal to 14.7 degree days and under short days the value was equal to 9.2 degree days (Table 5). The accumulated thermal units and photo-thermal units measured in degree days (ºCd) were computed for each accession by multiplying the derived thermal units and photo-thermal units by the number of days to flowering from seedling emergence. Days to flowering was negatively correlated ($r = -0.80$; $P \leq 0.0001$) with cumulative photo-thermal units and thermal units (date not shown). Regression analysis revealed that days to flowering was a function of both cumulative thermal units and photo-thermal units described as:

Days to flowering = 549 (26) - 25.7 (1.4) x thermal units; and

Days to flowering = 138 (4.5) - 7 (0.37) x photo-thermal units.

*Values in brackets represent the standard error.

This finding supports the fact that longer days hastened flowering time in the chickpea accessions compared to the short days because of the accumulation of greater amount of thermal and photo-thermal units under long days compared with short day photoperiods.

Table 3.3. Photo-thermal (PthU) and thermal units (TU) in degree days under long day (LD) and short day (SD) photoperiod treatments.

Temperature (in ˚C, day/night)	PthU		TU	
	LD (16 hours)	SD (10 hours)	LD (16 hours)	SD (10 hours)
(22/16)	14.7	9.2	20	18.5

The photo-thermal and thermal units accumulated from emergence to flowering for the chickpea accessions evaluated for their flowering response under short and long days are presented in Appendix III.

Days to flowering was negatively correlated ($r = -0.80$; $P \leq 0.0001$) with cumulative photo-thermal units and thermal units (date not shown). Regression analysis revealed that days to flowering was a function of both cumulative thermal units and photo-thermal units described as:

Days to flowering = 549 (26) - 25.7 (1.4) x thermal units

and

Days to flowering = 138 (4.5) - 7 (0.37) x photo-thermal units

*Values in brackets represent the standard error.

This finding supports the fact that longer days hasten flowering time in the chickpea accessions compared to the short days because of the accumulation of more thermal and photo-thermal units under long days compared with short day photoperiods.

3.5 Discussion

The existence of substantial variation in days to flowering and node number of first flower appearance was detected under long and short-day photoperiod treatments on a diverse collection of chickpea accessions. The accessions were categorized into highly-photoperiod sensitive, photoperiod-insensitive (day-neutral), and intermediate response. Two out of the four accessions identified as photoperiod-insensitive were desi accessions, which originated from arid or semi-arid environments of India. Another kabuli accession identified as photoperiod-insensitive was also collected from arid or semi-arid environments of India. The intermediate accessions included 25 kabuli and 24 desi accessions originating from the arid or Mediterranean environments. The intermediate category consisted of 9 accessions collected from ICRISAT and 13 accessions originated from Iran.

The most photoperiod-sensitive accessions included kabuli and desi accessions adapted to cool temperate environments including western Canada. Six cultivars developed at the University of Saskatchewan were classified as highly photoperiod sensitive with the delay in number of days to flowering ranging from 44 to 66 days. Eight kabuli accessions collected from ICARDA and nine accessions originating from Iran were also highly sensitive to photoperiod. The day-neutral accessions were early flowering under both long and short-days, whereas the highly-photoperiod sensitive accessions were mostly late flowering. This finding is similar to previous reports that variability between day-neutral and photoperiod-sensitive accessions was related to early flowering in the former and late flowering in the latter (Roberts et al., 1985; Summerfield et al., 1998).

Photoperiod-insensitive germplasm is useful for the improvement of geographical adaptation in soybean because insensitivity was mostly associated with extreme earliness (Cheng et al., 2011). Development of breeding lines with reduced photoperiod sensitivity is

expected to contribute to wider adaptation of chickpea to the short growing season of western Canada.

Time to first flower appearance was associated with the time taken to reach a particular vegetative stage (Roberts et al., 1985). The transition from the vegetative to the flowering stage is essential for survival, and plants normally time the onset of flowering to suitable environmental conditions (Dwivedi et al., 2008). Mei et al. (2009) reported a positive and significant correlation between plant height and flowering time in *Brassica napus*. Similarly, Anbessa et al. (2006) reported a positive association between time to flowering and plant height due to more vegetative growth in late flowering chickpea genotypes. Jamalabadi et al. (2013) characterized $F_{2:3}$ populations developed by crossing ILC 3279 x ICCV 2 for evaluation of genes governing time to flowering and plant height and reported positive correlation between plant height and days to flowering in lines with a similar genetic background to ICCV 2, the early flowering parent.

Introduction of photoperiod insensitivity into wheat cultivars brought about a significant reduction in height and early flowering (Worland, 1996). Plant size at maturity is of practical and theoretical interest because of its relationship to crop yields (Trudgill et al., 2005). Shorter plants bear fewer pods per reproductive tissues, which would generally lead to lower yields. Shorter plant stature could also cause difficulty in mechanical harvest.

Inferences about the relative times of flower initiation in a group of plants by noting the node at which flowering first occurs dates back several decades as reported by Aitken (1955) in subterranean clover (*Trifolium subterraneum* L.), Paton and Barber (1955) in field pea. They indicated that lower nodes of first flowering indicated earliness or rapid development whereas higher nodes of first flower were considered as indicative of lateness or slow development. Roberts et al. (1985) reported that flowers appeared at higher nodes in

chickpea exposed to 12 hours photoperiod compared to lower nodes with 15 hours photoperiod treatment. The same authors indicated an association of early flowering and maturity with node of first flower. In *Celosia*, (*Celosia argentea* L. var**.** *plumosa* Voss.) a facultative short-day plant, node of first flower was significantly reduced when a plant originally grown under non-inductive long day photoperiod for 6 days after emergence was exposed to short day (9 hours) photoperiod conditions (Warner, 2009).

On the other hand, floral buds could be initiated at any plant node under sufficiently short photoperiods to initiate flowering in soybean varieties (Zhang et al., 2001). Initiation of flower buds at different nodal positions in common bean (*Phaseolus vulgaris* L.) cultivars under different photoperiod conditions were attributed to rate of photosynthate partitioning in the crop. Wallace et al. (1993) reported preferential partitioning of the photosynthate to continue growth of additional stems, branches, and leaves which hinders early flower bud initiation at lower nodes in common bean. Roberts et al., (1985) concluded that except in very early flowering chickpea genotypes, the rate of vegetative growth and reproductive growth follows quite distinct processes independent of one another.

Thermal time has been used by biologists to analyse the effects of temperature on various developmental processes of plant species (Trudgill et al., 2005). Soltani et al. (2004, 2006) used data from four chickpea cultivars serially grown under field conditions and reported that the response of development rate to temperature and photoperiod was described by a multiplicative model with a dent-like function for response to temperature, and quadratic function for response to photoperiod. This research was conducted under two photoperiod treatments and constant mean temperature under long and short day photoperiods in the controlled conditions. When chickpea is sown in summer under field conditions, the increase in temperature and photoperiod occurs during the vegetative growth of the plant. Therefore, it

is difficult to avoid the confounding effect of temperature and photoperiod on flowering in chickpea genotypes (Roberts et al., 1985). In this research cumulative thermal units and photo-thermal units were perfectly correlated with days to flowering in the growth chamber experiments because temperatures were constant during each day of the experiment similar to finding by Holland et al. (2002).

Days to flowering in chickpea was significantly reduced with faster accumulation of both photo-thermal and thermal units. This is similar to the results of Trudgill et al. (2005) who reported that rapid development results from a small overall degree day increase in diverse plant species with short generation times. Further, a regression analysis in our study revealed that days to flowering was a function of thermal units and photo-thermal units in chickpea. Flowering time is regulated by balancing the promotive or inhibitory effects of different environmental conditions (Reeves and Coupland, 2000). Higher cumulative photo-thermal and thermal units facilitated the induction of flowering and inhibited vegetative growth compared with lower cumulative photo-thermal and thermal units because rate of development has a linear relation to temperature in plants (Summerfield et al., 1991; Trudgill et al., 2005). Summerfield and Roberts (1988) reported that progress to flowering is a positive linear function of mean temperature between a critical minimum base to an upper optimum, above which rates decline again until the maximum temperature at which flowering can occur. Under the anticipated global warming, temperature sensitive cultivars will flower relatively earlier compared to those responding largely to photoperiod, supporting the exploitation of the available diversity in developing well-adapted genotypes for emerging cropping environments (Berger et al., 2011; Vadez et al., 2012).

Purushothaman et al. (2014) reported that the longer reproductive and shorter vegetative phase in kabuli chickpea accessions lead to less total shoot biomass production

which in turn resulted in lower harvest index and grain yield under drought conditions in Patancheru, India. Kabuli germplasm require more time for their reproductive phase under cooler environments because they have evolved in an environment where the later part of the pod-filling period occurs in a constantly warming environment that forces maturity through rapidly increasing degree days (Purushothaman et al., 2014). Habitats that impose high terminal drought stress are favouring early flowering and a short lifecycle as a drought escape mechanism, whereas cool, high rainfall habitats select for delayed phenology (Nelson et al., 2010). Chickpea accessions originating from India were early to flower and were the least responsive to photoperiod (Roberts et al., 1985).

In conclusion, the current experiments demonstrated variability in days to flowering in the diverse chickpea accessions grown with long and short day photoperiod. The chickpea accessions originating from the tropics and low latitudes were identified as photoperiod-insensitive, and those from diverse geographical origins were intermediate to photoperiod sensitive. Chickpea accessions adapted to cooler, higher latitude environments were photoperiod sensitive in their flowering response under the photoperiod regimes. Longer days hastened flowering time in chickpea compared to short days because of higher cumulative thermal and photo-thermal units under long days compared with short days.

4. Effect of Temperature and Photoperiod on Time to Flowering in Chickpea Accessions

Abstract

Flowering time is a key factor in determining the adaptation of crops to diverse environments. Temperature and photoperiod are the two major environmental variables that affect the length of the period between sowing and flowering and the rate of plant development. The objectives of this research were to examine the days to flowering of selected chickpea accessions grown in a range of thermal regimes combined with either long or short days, and to examine the interaction between photoperiod and day and night temperatures on days to flowering. Eight chickpea accessions representative of different photoperiod sensitivity responses were included, i.e., day-neutral (ICCV 96029 and FLIP-98-142C), intermediate (ICC 8621, ICC 8855, ICC 15294, and ILC 1687), and highly photoperiod-sensitive (CDC Frontier and CDC Corinne). Significant effects of accession, temperature, photoperiod, and their interaction were observed for days to flower. Earliest flowering was observed in day-neutral accessions followed by intermediate accessions, then photoperiod sensitive accessions which flowered on average in 20, 23, and 41 days, respectively, under long photoperiod combined with higher temperature regimes. For the two day-neutral accessions, the difference in the number of days to flower under 16 hours photoperiod combined with the temperature regimes of 24/16 ˚C and 20/12 ˚C were not significant. Regression analysis revealed that days to flower of the day-neutral, intermediate and photoperiod sensitive accessions was a linear function of temperature (R^2 = 0.88 to 0.99) within the photoperiod.

4.1 Introduction

Flowering time is crucial in determining the adaptation of crops to diverse environmental conditions (Tsubokura et al., 2013). Temperature and photoperiod are the two major environmental variables that affect the length of the period between sowing and flowering as well as the pace of plant development (Roberts et al., 1985; Yin, 2008). Flowering time, pod development and maturity, therefore, play critical roles in adaptation of chickpea cultivars to different environments (Anbessa et al., 2007b; Berger and Turner, 2007; Gaur et al., 2008). Temperatures greater than a critical minimum base to an upper optimum limit, long photoperiods, and moisture stress conditions all shorten developmental phases in plants, reducing the crop lifecycle (Vadez et al., 2012). With global climate change, crop duration in plant species is expected to be shorter (Wheeler et al., 1996; Vadez et al., 2012). Increased temperature affects the rate of crop development through increased accumulation of thermal time (Wheeler et al., 2000). This could bring about unanticipated alteration in crop production depending on the relative effects of photoperiod and ambient temperature on phenology.

Knowledge of the time required for crops to progress to flowering and other key phenological stages including pod set and maturity allows growers to fit crop lifecycles to the available cropping season (Gaur et al., 2008; Vadez et al., 2012). Early flowering and early pod setting and development restrict vegetative growth in indeterminate crops like chickpea (Saxena, 1988; Saxena, 1990; Anbessa et al., 2007a).

Chickpea was recently introduced to Canada and since then the area under chickpea production has been fluctuating. Due to the short growing season in western Canada (110-120 days), maturity may coincide with end-of-season frost and result in severe losses in grain yield and quality (Warkentin et al., 2003). Thus, it is essential to match crop duration with the

available growing season in order to minimize the yield penalty. In chickpea, time to flowering is a major component of crop duration and it is modulated by genotype, temperature, photoperiod, and their interactions (Roberts et al., 1985).

Temperature sensitivity in chickpea is strongly correlated with mean temperature during the vegetative phase in the habitat of origin (Berger et al., 2011). Appropriate phenology that minimizes exposure to climatic stresses and maximizes productivity in target environments is the most important adaptive criterion in annual crops (Gaur et al., 2008; Vadez et al., 2012). Understanding the photo-thermal effects on days to flower in diverse chickpea accessions would enable us to better bridge the gap between the current and desired level of earliness in chickpea in western Canada. Therefore, as the first step toward improving adaptation and grain yield, selecting for early flowering and early maturing cultivars should be a major objective in Western Canada to develop short-season cultivars. Our first objective was to examine the flowering response of selected chickpea accessions grown in a range of thermal regimes combined with either long or short-days, and the second was to examine the interaction between photoperiod and day and night temperatures on crop duration.

4.2 Materials and Methods

Research was conducted in the growth chambers at the College of Agriculture and Bioresources, University of Saskatchewan. The first time replicate of the experiment was completed in 2012 and the second time replicate was completed in 2013. Factorial combinations of each of two photoperiods, long days (16 and 8 hours day and night, respectively) and short days (10 and 14 hours day and night, respectively), with each of three diurnal temperature regimes (24/16 ºC, 20/12 ºC, and 16/8 ºC day/ night) (Appendix IV) (in a completely randomized experimental design within a growth chamber) were imposed on eight diverse chickpea accessions collected from gene banks of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India and International Center for Agricultural Research In the Dry Areas (ICARDA), formerly at Syria, together with cultivars developed at the Crop Development Centre (CDC) of the University of Saskatchewan as indicated in Table 4.1.

The eight accessions were selected based on their flowering response under long and short day photoperiods in previous experiments conducted in the same facility. CDC Frontier (Warkentin et al., 2005), a kabuli cultivar, and CDC Corinne (Tar'an et al., 2009), a desi cultivar were developed by the Crop Development Centre, University of Saskatchewan and are sensitive to photoperiod. Accessions with intermediate response to photoperiod had diverse geographical origins and included ICC 8855 and ILC 1678 kabuli accessions originating from Afghanistan, ICC8621 and ICC 15294 desi accessions originating from Ethiopia and Iran, respectively. The two day-neutral accessions included ICCV 96029, a desi accession from ICRISAT, and FLIP-98-142C, a kabuli accession from ICARDA. For simplicity, all genotypes evaluated will be referred to as 'accessions'.

Three seeds of individual accessions were grown in a 7.6 L pots containing Sunshine mix media #4 (Sun Gro, Seba Beach, AB). Two weeks after seeding and after full emergence, the seedlings were thinned to two plants per pot. Eight replicates of each accession were grown in each chamber. One chamber represented one treatment combination of one temperature regime and photoperiod. Relative humidity of 70 ± 5% was maintained in each chamber. Plants were watered every 2 to 4 days depending on crop growth stage and corresponding water use. Once a week a quick release fertilizer (20 N:20 P_2O_5:20 K_2O) prepared at a concentration of 3 g/l was applied at a rate of 100 ml per pot starting one week after emergence. Illumination was provided by fluorescent cool white light bulbs (T5 HO 835; 3500k) with constant radiation flux density of 370 µmol $m^{-2}s^{-1}$. Since in natural conditions, the diurnal change in air temperature lags asymmetrically behind that of radiation, the day temperatures in the cabinets were set to start 2 hours after the lights came on and end at 'lights off' following the procedure described by Roberts et al. (1985).

Table 4.1. Name of accession, market class and categories in their response to photoperiod of eight chickpea accessions representative of desi and kabuli market classes evaluated in a factorial combination of two photoperiods (16 and 10 hours) and three temperature regimes(24/16 ˚C, 20/12 ˚C, and 16/8 ˚C).

Accessions	Market class	Origin	Potential photoperiod sensitivity categories
CDC Frontier	Kabuli	CDC	Photoperiod-sensitive
CDC Corinne	Desi	CDC	Photoperiod-sensitive
ICCV 96029	Desi	ICRISAT	Day-neutral
FLIP- 98-142C	Kabuli	ICARDA	Day-neutral
ICC 15294	Desi	Iran	Intermediate
ILC 1687	Kabuli	Afghanistan	Intermediate
ICC 8855	Kabuli	Afghanistan	Intermediate
ICC 8621	Desi	Ethiopia	Intermediate

4.3 Data Analyses

Data were recorded for the number of days to flower bud initiation (number of days from seeding to the appearance of the first flower bud) and number of days to flowering (number of days from seeding to the appearance of the first fully open flower). In some accessions which were grown under less favorable treatments (lower temperature regime combined with short days), continuous vegetative growth occurred before the onset of flowering which caused overcrowding in the growth chambers. For these accessions, two pots per genotype were discarded and the number of days to flowering was scored on the remaining six pots. Data from two plants from each pot were averaged and recorded as the number of days to flowering of the particular accession. The experiment was conducted twice with six replicates in each time replicate. There was no significant effect of time replicate, so data were pooled over the time replicates. Data were analyzed with a three-way factorial ANOVA model, with accession, mean temperature and photoperiod as main factors. Fisher's protected LSD test was used for mean separation. All ANOVA and mean separation calculations were performed using PROC GLM in SAS 9.3 (SAS Institute Inc., Cary, NC) for completely randomized experimental design. Differences among the categories of the chickpea accessions for their response to flowering under temperature and photoperiod were detected using PROC CONTRAST and the response of accessions to changes in temperature and photoperiods was determined using PROC REG of SAS 9.3 (SAS Institute Inc., Cary, NC). Significant difference among the treatments was revealed at ($P \leq 0.05$).

Various forms of temperature summations, commonly referred to as thermal units or growing degree days, have been utilized in numerous studies to predict phenological events for agronomic and horticultural crops. Thermal time calculations usually involve three cardinal temperatures: (i) a base temperature (T_b) below which no development occurs, (ii) an optimum temperature (T_o) at which development proceeds at a maximum rate and (iii) a

maximum temperature (T_{max}) above which no development occurs. In our study, because the experiment was conducted under uniform day and night temperatures in the growth chamber and the switch to the nominal day temperature was delayed for 2 hours after the start of the photoperiod, daily thermal units (T_u) were calculated following a procedure similar to Summerfield and Roberts (1988) and Summerfield et al. (1985). In this study, the diurnal temperatures were adjusted to be constant and T_b was not included in the calculation of the Thermal Units.

Thermal units = [(Day hours –2) x T_{max}] + [(night hours +2 x T_{min})]/24 hours] –T_b)

Cummultive thermal units = [(Day hours –2) x T_{max}] + [(night hours +2 x T_{min})]/24 hours] – T_b) x days to flowering.

Where T_{max} and T_{min} are maximum and minimum daily air temperatures and T_b is the base temperature below which development ceases.

Similarly, photo-thermal units were calculated taking into consideration the fact that the switch to the nominal day temperature was delayed for 2 hours after the start of the photoperiod (light on).

Photo-thermal units = [(day hours -2) x day temperature)] +2 hours x night temperature).

Cummulative Photo-thermal units = [(day hours -2) x day temperature)] +2 hours x night temperature)/day hours x days to flowering.

4.4 Results

Analysis of variance was conducted for accession, mean temperature and photoperiod as main factors. Across the accessions, higher temperature and longer photoperiod were effective in hastening flowering; however, there was a signficant interaction between these two factors (Table 4.2).

Table 4.2. Analysis of variance of the effect of accessions, photoperiod, temperature, and their interaction on number of days from seedling emergence to first flower appearance of eight chickpea accessions grown in a combination of two photoperiod and three temperature regimes replicated over two time replicates.

Source of variation	df	F-Value
Accessions	7	3789.2***
Photoperiod	1	12264.3***
Temperature	2	5852.2***
Accession x Photoperiod	7	383.4***
Accession x Temperature	14	176.7***
Photoperiod x Temperature	2	342.8***
Accession x Photoperiod x Temperature	14	38.8***

df = degrees of freedon, *** indicates, highly significant different at $P \leq 0.0001$, $R^2 = 0.99$, CV= 5.39, RMSE = 2.65, Mean days to flowering = 50 days

4.4.1 Effect of photoperiod on days to flowering

A significant ($P \leq 0.0001$) effect of photoperiod on the days to flowering was detected for the chickpea accessions. On average, days to flowering were hastened under longer day conditions as compared to the shorter days. The least square means for days to flowering of all accessions were 38 and 61 under long (16 hours) and short day conditions (10 hours), respectively.

4.4.2 Effect of temperature regime on days to flowering

Significant ($P \leq 0.0001$) differences among the three temperature regimes were detected in their effect on days to flowering of the chickpea accessions. On average, earlier flowering was observed under higher temperature regimes compared to lower temperature regimes. The least square means for days to flowering of all accessions were 37, 46, and 64 days under 24/16 ºC, 20/12 ºC, and 16/8 ºC, mean temperatures regimes, respectively.

4.4.3 Interaction of temperature, photoperiod and accession on days to flower

There was no significant difference in mean number of days to flowering, in terms of the mean of all accessions, under a combination of temperature and photoperiod between the two experimental time replicates (50 days in both time replicates). Analysis of variance was conducted for accession, mean temperature and photoperiod as main factors. Significant ($P \leq 0.0001$) accession, temperature and photoperiod interactions were detected for days to flowering across the selected chickpea accessions (Table 4.3). The flowering response of accessions varied from early flowering for day-neutral accessions, to late flowering for photoperiod sensitive accessions. The intermediate accessions had medium days to flowering. The range varied from 26 days for ICCV 96029 an early flowering and day-neutral accession, to 75 days for CDC Frontier, a late flowering and highly photoperiod sensitive accession.

Table 4.3. Average number of days to flowering of eight chickpea accessions representative of desi and kabuli market classes evaluated in a factorial combination of 2 photoperiods (16 and 10 hours) and temperature regimes (24/16 ºC, 20/12 ºC, and 16/8 ºC) in two time replicates.

Accessions	Market class	Mean	*N	Potential categories
CDC Frontier	Kabuli	75[a]	72	Photoperiod-sensitive
CDC Corinne	Desi	72[b]	72	Photoperiod-sensitive
ICC 15294	Desi	54[c]	72	Intermediate to photoperiod
ILC 1687	Kabuli	48[d]	72	Intermediate to photoperiod
ICC 8621	Desi	45[e]	72	Intermediate to photoperiod
ICC 8855	Kabuli	42[f]	72	Intermediate to photoperiod
FLIP- 98-142C	Kabuli	30[g]	72	Day-neutral
ICCV 96029	Desi	26[h]	72	Day-neutral

*N= number of values for each genotype used in the analysis (6 plant samples, evaluated under 2 photoperiods and 3 diurnal temperature regimes over 2 time replicates); means values with the same letter are not significantly different based on LSD ≤ 0.05.

4.4.4 Comparison among the photoperiod response categories

Orthogonal contrasts were made using the mean number of days to flower for each accession evaluated under the two photoperiod and the three diurnal temperature regimes. Significant differences were detected among the photoperiod response categories (Table 4.4). The differences in number of days to flower in photoperiod sensitive vs. day-neutral, photoperiod sensitive vs. intermediate, day-neutral vs. intermediate, photoperiod-sensitive and intermediate vs. day-neutral, photoperiod sensitive vs. intermediate and day-neutral categories were all highly significant ($P \leq 0.0001$). The effect of the three temperature regimes on days to flower in the three photoperiod categories was also highly significant. The overall average number of days to flower for day-neutral, intermediate and photoperiod- sensitive categories were 27, 46 and 74 days, respectively.

4.4.4.1. Photoperiod insensitive (day-neutral) accessions

Comparisons of flowering response of chickpea accessions to changes in temperature regimes within the same photoperiod, and the same diurnal temperature regime across different photoperiods indicated that there were no significant differences for days to flowering for ICCV 96029 a day-neutral accession under long vs. short days combined with higher diurnal temperature regimes (24/16 ºC and 20/12 ºC) (Table 4.5). However, there was a significant ($P \leq 0.0001$) difference in the number of days to flowering at lower temperature regimes (16/8 ºC) under long versus short day photoperiods. This accession flowered in 28 under long days and 38 days under short days combined with lower temperature (16/8 ºC).

Table 4.4. Response of chickpea accessions to three temperature regimes (24/16 ºC, 20/12 ºC, and 16/8 ºC), two photoperiods (16 hours (LD) and 10 hours (SD), and the interaction of temperature and photoperiod.

Temperature x Photoperiod	Categories of accessions		
	Day-neutral	Intermediate	Photoperiod-sensitive
12 ˚C vs. 16 ˚C and 20 ˚C	***	***	***
12 ˚C at SD vs. LD	***	***	***
16 ˚C at SD vs. LD	NS (1)	***	***
20 ˚C at SD vs. LD	NS (2)	***	***
12 ˚C vs. 16 ˚C at SD	***	***	***
16 ˚C vs. 20 ˚C at SD	***	***	***
12 ˚C vs. 20 ˚C at SD	***	***	***
12 ˚C vs. 16 ˚C at LD	***	***	***
16 ˚C vs. 20 ˚C at LD	***	***	***
12 ˚C vs. 20 ˚C at LD	***	***	***

*** indicates significant difference at $P \leq 0.0001$, NS = not significant, (1) Only ICCV 96029 and (2) Both ICCV 96029 and FLIP -98-142C.

There was no significant difference in the number of days to flowering for FLIP-98-142C under long and short days combined with a diurnal temperature of (24/16 ºC); however, there was significant ($P \leq 0.0001$) difference in the number of days to flowering under long and short days combined with lower diurnal temperature regimes (20/12 ºC and 16/8 ºC). FLIP-98-142C flowered in 21 and 22 days under long and short days combined with 24/16 ºC diurnal temperature, respectively, while it flowered in 27 and 31days under long and short days combined with mean temperature of 20/12 ºC. This accession flowered in 33 days under long-days combined with lower temperature and 45 days under short-days combined with lower temperature (16/8 ºC).

4.4.4.2 Intermediate accessions

The four intermediate accessions, (ICC 8621, ILC 1687, ICC 15294, and ICC 8855), flowered earlier under long days and diurnal temperature regimes of 24/16 ºC compared to short days and/or cooler temperatures (Table 4.5).

4.4.4.3 Photoperiod-sensitive accessions

In the two photoperiod sensitive accessions, there was a significant ($P \leq 0.0001$) difference in number of days to flowering under short and long days combined with the three mean temperature regimes (24/16 ºC, 20/12 ºC, and 16/8 ºC). In these accessions, flowering was hastened by warmer temperatures under longer days. Significant delays in the number of days to flowering were observed under short days combined with lower temperature regimes. CDC Frontier flowered in 39, 52 and 91 days under long days combined with 24/16 ºC, 20/12 ºC, and 16/8 ºC diurnal temperatures, respectively. Days to flowering for this accession under short days combined with 24/16 ºC, 20/12 ºC, and 16/8 ºC diurnal temperatures were 67, 84 and 120 days, respectively.

Table 4.5. Days to flowering of eight diverse chickpea accessions as affected by the interaction between temperature regimes and photoperiods. Values within a column and within accessions for LD and SD followed by different letters are significantly different at $P \leq 0.05$.

Accessions		ICCV 96029		FLIP-98-142C		ICC 8621		ILC 1687		ICC 15294		ICC 8855		CDC Corinne		CDC Frontier	
Photoperiod		LD	SD	LD	SD	LD	SD	LD	SD	LD	SD	LD	SD	LD	SD	LD	SD
Temperature regimes (Day and night in ºC)	24/16	18^{d}	19^{d}	21^{d}	22^{d}	26^{f}	41^{d}	26^{e}	52^{c}	24^{e}	56^{c}	22^{d}	46^{b}	43^{e}	70^{c}	39^{f}	67^{d}
	20/12	25^{c}	26^{c}	27^{c}	31^{b}	36^{e}	48^{b}	33^{d}	62^{b}	35^{d}	66^{b}	29^{c}	49^{b}	60^{d}	84^{b}	52^{e}	84^{c}
	16/8	28^{b}	38^{a}	33^{b}	45^{a}	44^{c}	73^{a}	34^{d}	79^{a}	37^{d}	88^{a}	32^{c}	75^{a}	75^{c}	106^{a}	91^{b}	120^{a}

LD = Long Day (16 /8 hours day and night), SD = Short Day (10 /14 hours day and night); Mean values of the number of days to flowering followed by the same letters in the same columns indicate non-significant difference based on LSD at $P \leq 0.05$.

CDC Corinne flowered in 43, 60 and 75 days under long days combined with 24/16 ºC, 20/12 ºC, and 16/8 ºC diurnal temperatures, respectively. Days to flowering for CDC Corinne under short days combined with 24/16 ºC, 20/12 ºC, and 16/8 ºC temperature regimes were 70, 84, and 106 days, respectively.

4.4.5 Flowering response to temperature and photoperiod

The flowering response to photo-thermal conditions across the chickpea accessions can be described as a linear function of temperature. The regression equations were strong with the R^2 values ranging from 0.88 to 0.99 for the eight accessions (Figure 4.1). The relationship was described by the equation: Number of days to flower = y-intercept-Slope coefficients x temperature.

For all accessions, greater y-intercept and slope coefficient values were observed under short day photoperiods as compared to long day photoperiod and diurnal temperature treatment combinations (Table 4.6). Under short days, the slope of the regression line for day-neutral accessions ranged from -2.56 to -2.94, in intermediate accessions the slope coefficients were between -3.25 and -4.35, and in photoperiod sensitive accessions they ranged from -4.42 to -6.63. Under long days, the slope of the day-neutral accessions ranged from -1.19 to -1.48, in intermediate accessions the value was between -1.02 and -4.15 and in photoperiod sensitive accessions -3.85 to -6.33. Under short days, the y-intercept of the day-neutral accessions ranged from 69 to 80 days, 109 to 134 days in intermediate accessions and from 157 to 197 days in the photoperiod sensitive accessions. Similarly, under short days, the y-intercept of the day-neutral accessions ranged from 43 to 51 days, 47 to 104 days in intermediate accessions and 120 to 161 days in photoperiod sensitive accessions. Greater absolute values of slope and y-intercepts corresponded with late flowering and photoperiod sensitivity, whereas smaller absolute values of slopes and y-intercepts were associated with earliness or photoperiod insensitivity.

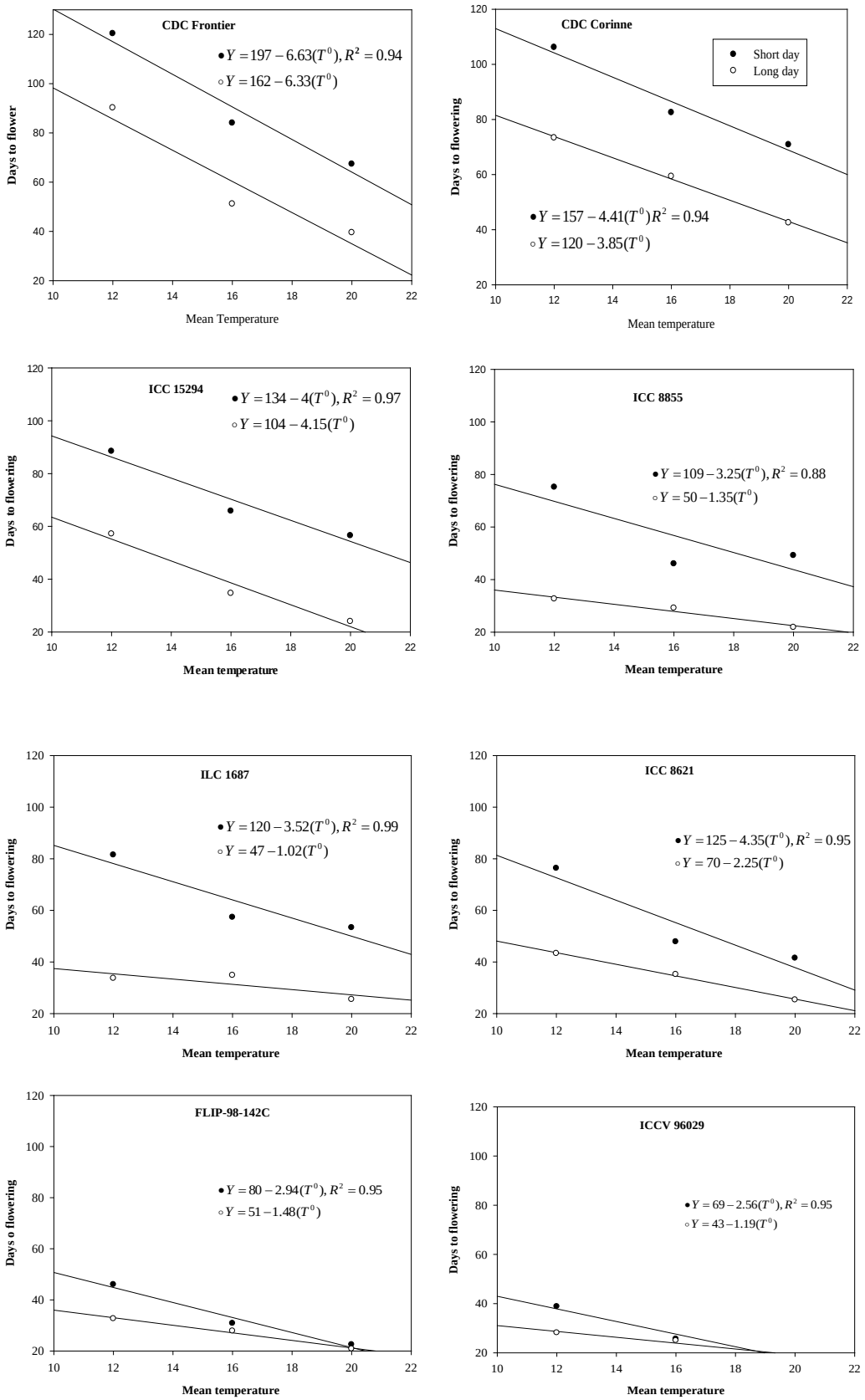

Figure 4.1. Regression analysis of the number of days from emergence to flowering to three temperature regimes (24/16 ºC, 20/12 ºC, and 16/8 ºC) under two photoperiod treatments (16 hours light/8 hours dark and 10 hours light /14 hours dark) across eight chickpea accessions (CDC Frontier, CDC Corinne, ICC 15294, ICC 8855, ILC 1687, ICC 8621, FLIP-98-142C, and ICCV 96029).

Table 4.6. Summary of the intercepts and the slope coefficients (photoperiod-sensitivity) for the eight diverse chickpea accessions evaluated under short-day and long-day conditions and three temperature regimes.

	Short-days		Long-days		CV (%)	R^2 (%)
	Intercept	Slope coefficients	Y-intercept	Slope coefficients		
Accessions						
CDC Corinne	157	-4.4	120	-3.9	7.1	0.94
CDC Frontier	197	-6.6	161	-6.3	8.8	0.94
FLIP 98-142C	80	-2.9	51	-1.5	6.6	0.95
CCV 96029	69	-2.6	43	-1.2	5.3	0.96
ICC 15294	134	-4.0	104	-4.2	7.7	0.97
ICC 8621	125	-4.4	70	-2.3	9.5	0.95
ICC 8855	109	-3.3	50	-1.4	14.6	0.88
ILC 1687	120	-3.5	47	-1.0	4.3	0.99

CV= coefficients of variation, R^2 = variability explained by the regression equation

4.4.6 Thermal and Photo-thermal units required for flowering of chickpea accessions

The integrated effect of both photoperiod/light duration and temperature (Photo-thermal units) or overall temperature regime combined with either long or short days (thermal units) regime alone on time to flowering in chickpea accessions was assessed by separate analysis for each unit.

4.4.6.1 Photo-thermal units for days to flower

Various forms of temperature summations, commonly referred to as thermal units or growing degree days, have been utilized in numerous studies to predict the phenological events for agronomic and horticultural crops. The photo-thermal units accumulated from emergence to flowering for the chickpea accessions evaluated under the three diurnal temperature regimes combined with long or short days are indicated in Table 4.7. Under a factorial combination of three temperature regimes and two photoperiods, the average accumulated photo-thermal units required from days to emergence to first flowering in the day-neutral accessions were 500 degree days, followed by 820 degree days for intermediates, and 1300 degree days for highly photoperiod sensitive accessions. In the two day-neutral accessions, ICCV 96029 and FLIP-98-142C, the highest values of the accumulated photo-thermal units were 544 and 644 degree days, respectively, under short days combined with 16/8 ˚C diurnal temperatures regime. For these accessions, the lowest value of the accumulated photo-thermal units were 330 and 405 degree days, respectively, under short days combined with mean diurnal temperature of 24/16 ºC. A similar pattern was observed in the intermediate accessions where the highest photo-thermal unit was accumulated under short days combined with a diurnal temperature of 16/8ºC. Accordingly the highest values were 897 degree days for ILC 1687, 974 degree days for ICC 15294, 840 degree days for ICC 8621 and 827 degree days for ICC 8855. For CDC Frontier, the highest accumulated photo-thermal unit (1,713 degree days) was recorded under short days

combined with a diurnal temperature of 16/8 ºC and the lowest value (909 degree days) was under long days combined with a diurnal temperature of 24/16 ºC. Similarly, in CDC Corinne, the highest accumulated photo thermal unit (1486 degree days) was under short days combined with a diurnal temperature of 16/8 ºC and the lowest value (978 degree days) was under long days combined with a diurnal temperature of 24/16 ºC.

Table 4.7. Photo-thermal (PthU) units required for flowering in 8 diverse chickpea accessions evaluated across 3 diurnal temperature regimes (24/16 ºC, 20/12 ºC, and 16/8 ºC day and night) and 2 photoperiods (long days = LD (16/8 hours day and night) and short days = SD (10/14 hours day and night) averaged over two time replicates.

Temperature	Photoperiod	PthU	Cumulative photo-thermal units (degree days)							
			CDC Corinne	CDC Frontier	ILC 1687	ICC 15294	ICC 8621	ICC 8855	FLIP-98-142C	ICCV 96029
16/8	SD	14	1486	1685	1141	1239	1069	1052	644	544
20/12	SD	15	1238	1260	860	987	717	690	462	385
24/16	SD	18	1275	1212	960	1017	747	882	405	330
16/8	LD	19	1393	1713	640	1086	823	621	621	535
20/12	LD	22	1305	1126	766	763	774	642	612	550
24/16	LD	23	978	909	587	552	583	502	479	429
Overall average		19	1,279	1,318	826	941	786	732	537	462

Temperature = Temperature regimes (˚C day/night)

4.4.6.2 Thermal units for days to flower

The average accumulated thermal units required from days to emergence to first flower appearance in the day-neutral accessions were 430 degree days, 700 degree days for intermediates, and 1100 degree days for highly photoperiod sensitive accessions under the factorial combination of three temperature regimes and two photoperiods (Table 4.8). In FLIP-98-142C the accumulated thermal units ranged from 338 under short days combined with diurnal temperature of 24/16 ºC to 555 degree days under long days combined with 16/8 ºC diurnal temperatures regime. In ICCV 96029, the highest value was 479 degree days under long days combined with 16/8 ºC diurnal temperatures regime, whereas the lowest value was 275 degree days under short days combined with diurnal temperature of 24/16 ºC. In the intermediate accessions the highest accumulated thermal unit of 974 degree days was recorded under short days combined with a diurnal temperature of 16/8 ºC for ICC 15294, where the lowest value of 458 degree days occurred under long days combined with 24/16 ºC for ICC 8855. The highest accumulated thermal unit of 1533 degree days for the photoperiod sensitive accession, CDC Frontier, was recorded under long days combined with a diurnal temperature of 16/8 ºC, where the lowest value of 830 degree days was recorded under long days combined with a diurnal temperature of 24/16 ºC. Similarly, in CDC Corinne, the highest accumulated thermal units of 1247 degree days was highest under long days combined with a mean diurnal temperature of 16/8 ºC and the lowest value of 893 degree days was recorded under long days combined with a mean diurnal temperature of 24/16 ºC.

Table 4.8. Thermal units (TU) required for flowering in 8 diverse chickpea accessions evaluated across the 3 diurnal temperature regimes (24/16 °C, 20/12 °C, and 16/8 °C) day and night) and 2 photoperiods averaged over two time replicates.

Temperature	Photoperiod	TU	Cumulative thermal unit (degree-days)							
			CDC Corinne	CDC Frontier	ILC 1687	ICC 15294	ICC 8621	ICC 8855	FLIP-98-142C	ICCV 96029
16/8	SD	11	1168	1324	897	974	840	827	506	427
20/12	SD	13	1073	1092	745	856	622	598	401	334
24/16	SD	15	1062	1010	800	848	623	735	338	275
16/8	LD	17	1247	1533	572	972	737	555	555	479
20/12	LD	19	1127	972	662	659	668	554	529	475
24/16	LD	21	893	830	536	504	532	458	437	392
Overall average		16	1,095	1,127	702	802	670	621	461	397

Temperature = Temperature regimes (°C day/night).

4.5 Discussion

The first and second time replicates of the experiments gave virtually the same days to flower for each accession, temperature and photoperiod and their combinations; the overall mean number of days to flower of the accessions under a combination of temperature and photoperiod was 50 in both time replicates. Thus, the interaction effects of accession, photoperiod, and mean temperature on days to flower for each accession over the duplicate time replicates is discussed. Variability in flowering response and relative earliness of eight diverse chickpea accessions was assessed under a combination of three day/night temperature regimes and two photoperiod treatments. The accessions flowered earlier under long days and higher temperature as compared to under short days and lower temperature. The accessions flowered under temperature regimes ranging from 8 to 24 °C and photoperiods of 16 and 10 hours. Similar findings were reported by Roberts et al. (1985) and Verghis et al. (1999) who observed earlier flowering under 15 °C compared to 10 °C. Sethi et al. (1981) reported that chickpea flowered earlier under a combination of long-days with a temperature regime of 30/18 °C compared to short days combined with a similar temperature regime.

Days to flowering of the accessions was shortened by long photoperiod, higher temperature regime and increased photoperiod sensitivity of the accessions. Similarly, Sethi et al. (1981) reported that the flowering in chickpeas was accelerated under long days of 15 hours combined with warm temperature regimes of 30/18°C. Days to flower of chickpea accessions was described by a linear function of temperature. Greater values of y-intercepts and slopes were detected under short days compared to long. These values were higher for photoperiod sensitive accessions compared to the day-neutral. This agrees with Clerget et al. (2007) who indicated a strong relationship between late flowering and photoperiod-sensitivity in sorghum, with the earliest flowering varieties exhibiting a slope

of values near 0, and the latest flowering varieties with values approaching 1.0. Major (1980) also indicated that the slope of the response line obtained in non-optimal photoperiods provided an estimate of photoperiod sensitivity in nine crop species.

The difference among the chickpea accessions in their flowering response to a combination of photoperiod and temperature treatments revealed that the differences were related to their geographical area of adaptation. Berger et al. (2011) reported that germplasm origin of chickpea had a dramatic effect on the relationship between photoperiod and temperature response. In genotypes of Mediterranean origin, temperature insensitivity was compensated by a strong photoperiod response, while in those of Indian origins, temperature sensitivity decreased with increase in photoperiod-sensitivity (van der Maesen, 1972). The orderly change of photoperiod sensitivity in chickpea with latitude was discussed by several authors (Roberts et al., 1985; Summerfield et al., 1997; Or et al., 1999; Hovav et al., 2003), in lentil by Erskine et al. (1990) and in cowpeas by Craufurd et al. (1999) and Lush and Evans (1980). In pea *(Pisum sativum* L.**)** and lentil *(Lens culinaris* Medik.), early-flowering habit is often associated with photoperiod insensitivity (Erskine et al., 1990; 1994; Arumingtyas and Murfet, 1994). These reports in diverse legume species have demonstrated remarkable correspondences in their response to these factors.

Photoperiod-insensitive genotypes of bean flowered and matured earliest and attained a higher harvest index compared with the photoperiod-sensitive genotypes (Yourstone et al., 1993). Incorporation of genes for early maturity and photoperiod insensitivity into unadapted germplasm is a breeding target for crops such as spring wheat (Dyck et al., 2004) and common bean (Singh, 2001).

Genes controlling flowering time in chickpea are sensitive to temperature and change in latitude. In India, mean days to flowering of 51, 76 and 96 days for 25 chickpea germplasm accessions were recorded at latitudes of 18 ˚N, 26 ˚N, and 29 ˚N, respectively

(Kumar and Abbo, 2001). They also reported that an extra early flowering chickpea genotype ICCV 96029 flowered in 24 days and 43 days at two contrasting locations at 18 ºN and 29 ºN, respectively, indicating a significant influence of geographical location on time to flowering. A significant genotype, temperature and photoperiod interaction was reported in lentil with earlier flowering time under longer days combined with warmer temperatures (Summerfield et al., 1985).

In soybean, a short day plant, short day photoperiod combined with high temperatures brought about earlier flowering while low temperatures and long days caused late flowering (Liu et al., 2011). In Mediterranean environments, genotypic differences in sensitivity to temperature and photoperiod explained most of the variability in flowering behavior of eight diverse forage legumes: sulla (*Hedysarum coronarium* L.); sainfoin (*Onobrychis viciifolia* Scop.); pea (*Pisum sativum* L.); berseem clover (*Trifolium alexandrium* L.); Persian clover (*Trifolium resupinatum* L.); faba bean (*Vicia faba* L.); common vetch (*Vicia sativa* L.) and hairy vetch (*Vicia villosa* Roth.) (Iannucci et al., 2008).

The earlier flowering of the chickpea accessions under longer days combined with higher temperatures resulted from faster accumulation of the thermal sum required for flowering. Highest accumulated photo-thermal and thermal units were recorded under lower temperature regimes combined with short and long days because of maximum days from sowing to flowering under these environments. Similarly, Keeve et al. (2000) reported that earlier flowering occurred under warmer days and longer photoperiod than a certain minimum in white lupin under field conditions. Berry and Aitken (1979) reported that in an early flowering pea variety, days from sowing to first flower was inversely proportional to temperature with no effect of photoperiod.

The factorial combination of two photoperiods and three temperature regimes in this research allowed us to derive daily photo-thermal and thermal units range from 14 to 23 and 11 to 23 degree days, respectively. Flowering response of the chickpea accessions under changes in photo-thermal and thermal units indicated that longer photoperiod and higher temperature regimes (24/16 °C) facilitated earlier flowering.

The association between temperature and accumulation of thermal units continues only in environments where diurnally changing temperatures do not exceed a high temperature maximum (Summerfield et al., 1991). A significant relationship between thermal time and time to flowering was reported in chickpea with a mean photo-thermal time of 447 degree days above the base temperature of 4 ˚C required in the Mediterranean environments of New Zealand (Verghis et al., 1999).

Short crop growing duration of 110-120 days was reported for chickpea in western Canada (Warkentin et al., 2003). The current study determined that the average accumulated thermal units required from seedling emergence to first flowering ranged from 397 dergee days in ICCV 96029 to 1127 degree days in CDC Frontier across the temperature regimes and photoperiods. In Saskatchewan, growing degree days of 470 degree days to full flowering in chickpea were reported by Gan et al. (2006b) and 730 to 740 degree days by Miller et al. (2006).

Roberts et al. (1985) reported that there was no interaction effect of temperature and photoperiod on the rate of progress towards flowering in chickpea accessions. They conducted the experiment with a wider range of temperature regimes of 20/10, 25/15 and 30/20 °C and narrower range of photoperiod treatments 12/12 and 15/9 hours (day and night, respectively). They commented that the maximum photoperiod range used in their experiment could have prevented an interaction between photoperiod and temperature (Roberts et al., 1985). The current research was conducted in a controlled environment in

growth chambers with photoperiods of 10/14 and 16/8 hours and wider temperature ranges of (24/16, 20/12 and 12/8 °C, day/ night temperature). Our results indicated a significant interaction of temperature and photoperiod for time to flowering in selected chickpea accessions. The information generated on the interaction between accessions, photoperiod and temperature is crucial for varaietal development to the short growing invironments of western Canada.

In conclusion, significant variability exists in the chickpea accessions for their flowering response under a combination of temperature regime and photoperiod. Variation in time to flower among chickpea accessions under a combination of temperature and photoperiod was revealed with earlier flowering in the day-neutral accessions, medium flowering in the intermediate accessions and latest flowering in the highly photoperiod sensitive accessions. The variability among the chickpea accessions for their flowering response to temperature, photoperiod and their interactions could be associated with their adaptation to the geographic origin. Thus, the availability of a wider collection of germplasms containing genes influencing photoperiod, temperature and their interactions may assist chickpea breeders to produce better broadly adapted cultivars in western Canada.

5. Determination of Photoperiod-sensitive and Photoperiod-insensitive Phases in Selected Chickpea Accessions

Abstract

Photoperiod is one of the major environmental factors determining time to flower initiation and first flower opening in plant species. In chickpea, photoperiod sensitivity, expressed as delayed days to flower under short days as compared to long days, may change with the growth stage of the crop. Photoperiod-sensitive and insensitive phases can be identified by experiments in which individual plants are reciprocally transferred in a time series from long to short-days in growth chambers. Eight chickpea accessions with differing degrees of photoperiod sensitivity were grown in two separate chambers, one of which was adjusted to long-days (16 hours light/8 hours dark) and the other adjusted to short-days (10 hours light/14 hours dark), with temperatures of 22/16°C (light/dark) in both chambers. The accessions included day-neutral (ICCV 96029 and FLIP-98-142C), intermediate (ICC 15294, ICC 8621, ICC 8855, and ILC 1687), and photoperiod-sensitive (CDC Frontier and CDC Corinne) responses. Control pots were grown continuously under the respective photoperiods. Reciprocal transfers between the short and long-day photoperiod treatments were made at seven time points after sowing, customized for each accession based on previous data. Photoperiod sensitivity was detected in intermediate and photoperiod sensitive acceessions. On average a 10-day difference was detected in the number of days to flower under short days compared with long days and subsequent transfers in day-neutral accession FLIP-98-142C, whereas there was no significant difference in ICCV 96029. In photoperiod-sensitive accessions, three different phenological phases were identified: a photoperiod-insensitive pre-inductive phase, a photoperiod-sensitive inductive phase, and a photoperiod-insensitive post-inductive phase. The photoperiod-sensitive inductive phase extended from after flower

initiation to full flower development. Results from this research will help to develop cultivars with shorter pre-inductive photoperiod-insensitive and photoperiod-sensitive phases to fit the cool, short growing season of western Canada.

5.1 Introduction

In order to maximize crop yield through agronomic management or plant breeding it is essential to ensure that the phenology of the crop is well matched to the resources and constraints of the production environment (Summerfield et al., 1996). All plants undergo several developmental transitions during their life cycle which can be divided into three major physiological developmental phases: vegetative development phase, from emergence to flower initiation, the reproductive development phase, from floral initiation to anthesis, and physiological maturity, from anthesis to seed filling (Ritchie, 1991; Ritchie et al., 1998). The vegetative growth phase is comprised of the basic vegetative phase and the photoperiod-sensitive phase (Vergara and Chang, 1985).

The transition from vegetative to reproductive phase is a major developmental switch in the plant's life cycle (Levy and Dean, 1998). This transition is crucial for survival because plants normally time the onset of flowering to suitable environmental conditions. Many plant species have thus evolved the ability to initiate flowering in response to environmental effects such as changes in photoperiod and temperature. The beginning stage of flowering is the visible start of the seed set period, and thus a key stage in yield formation (Lejeune-Hénaut et al., 1999; Putterill et al., 2004). Flower development and the seed set stages are greatly impeded by stress, such as drought and frost, thus flowering and seed development must be completed during favorable growing conditions. Timely flowering and maturity in relation to the available growing season in a particular location are essential for large potential yields from annual crops (Bunting, 1975). Thus, for proper crop management, understanding the photoperiod sensitive-phase of a photoperiodic plant would allow growers to either promote early flowering to reduce crop duration time, or intentionally delay flowering (Warner, 2009).

Crop development is a sequence of phenological events that determine the changes in the morphology as well as function of organs (Slafer and Rawson, 1994). The basic vegetative phase is the duration of vegetative growth under the optimum photoperiod whereas the photoperiod-sensitive phase is the growth period beyond the basic vegetative phase when the prevailing photoperiod is longer than the optimum photoperiod to initiate flowering (Vergara and Chang, 1985).

In experiments where transfers were made between two photoperiods for rice cultivars, the photoperiod-sensitive phase was flanked by two photoperiod-insensitive phases (Yin, 2008). In wheat, a long-day plant, the full pre-anthesis period was found to be divided into three sub-phases: from sowing to the terminal spikelet, from terminal spikelet initiation to heading, and from heading to anthesis indicating stage-dependence of plant responsiveness to temperature (Slafer and Rawson, 1995). In this crop, flowering response is affected by temperature throughout their life cycles (Slafer and Rawson, 1994). In short-day crop species such as cowpea and soybean, there was a temperature-dependent critical photoperiod. Beyond the critical point, time to flowering was solely a function of mean temperature (Hadley et al., 1984). Maize is sensitive to photoperiod at the stage of tassel initiation (Kiniry et al., 1983). In rice and soybean, the photoperiod influence extends for some time beyond the phase of floral initiation (Collinson et al., 1992; Ellis et al., 1992).

Earlier studies on wheat reported that exposure to long photoperiods significantly reduced the time to heading (Slafer and Rawson, 1997). Estimation of phasic development is crucial for accurate modelling of plant development and yield components, as well as for evaluating cultivar adaptation and scheduling cultural practices (Shaykewich, 1995). Quantitative models to determine phasic development phases in different plants were developed by different authors using different parameters and plant materials. Flower development phases were quantified using four parameters; a_1 (the photoperiod-insensitive

pre-inductive phase), I_s (the photoperiod-sensitive inductive phase in long and short-days), and a_3 the photoperiod-insensitive post-inductive phases in long and short-days (Ellis et al., 1992). Similarly, photoperiod-sensitive inductive phases in long day (LD) and short days (SD) were denoted as I_{2L} and I_{2S}, respectively, by following the procedure developed by Yin (2008).

Short-days will not delay flowering in a long day plant if exposure is restricted to the photoperiod-insensitive pre-inductive phase or the photoperiod insensitive phase of flower development. However, time to flower is delayed if the plant is exposed to short-days during the photoperiod-sensitive phase. Similarly, long-days will only hasten flowering in long-day plants if the plants are exposed to the photoperiod when they are at the photoperiod-sensitive stage (Adams et al., 2001). The duration of the photoperiod-sensitive phases can be determined by examining data on the time to first flower opening of plants transferred between short and long days at different times (Adams et al., 2001; Wang et al., 1997; Yin et al., 1997). Little is known about the duration of the photoperiod sensitive and insensitive phases in chickpea. Thus, the reciprocal transfer technique could be used to quantify and identify the timing and duration of the photoperiod-sensitive phase and the time of floral initiation in chickpea. The hypothesis of this study was that a photoperiod-sensitive phase exists in chickpea. The objectives of this research were to determine the timing and duration of the photoperiod-sensitive and photoperiod insensitive phases in selected chickpea accessions representative of different maturity classes, and to establish whether photoperiod sensitivity ends at floral initiation or extends into the phases of flower development.

5.2 Materials and Methods

Eight diverse chickpea accessions, namely ICCV 96029, FLIP-98-142C, ICC 8621, ILC 1687, ICC 15294, ICC 8855, CDC Corinne, and CDC Frontier collected from gene banks of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India and the International Center for Agricultural Research in the Dry Areas (ICARDA), together with cultivars developed at the Crop Development Centre, University of Saskatchewan were used in this research (Table 5.1). These eight genotypes will be referred to as 'accessions' throughout this chapter.

Table 5.1. Name, market class and photoperiod response categories of the eight chickpea accessions evaluated in a reciprocal transfer approach from long to short day photoperiod conditions.

Accessions	Market class	Origin	Potential photoperiod sensitivity categories
CDC Frontier	Kabuli	CDC	Photoperiod-sensitive
CDC Corinne	Desi	CDC	Photoperiod-sensitive
ICCV 96029	Desi	ICRISAT	Day-neutral
FLIP- 98-142C	Kabuli	ICARDA	Day-neutral
ICC 15294	Desi	Iran	Intermediate
ILC 1687	Kabuli	Afghanistan	Intermediate
ICC 8855	Kabuli	Afghanistan	Intermediate
ICC 8621	Desi	Ethiopia	Intermediate

The accessions were grown in two separate growth chambers: one of the chambers was adjusted to short days of 10 hours (SD) and the other chamber was adjusted to long days of 16 hours (LD). The chambers were maintained at day/night temperatures of 22/16 °C (12/12 hours). The 12/12 hours cycle was used to avoid confounding effects of an asynchrony between thermal and photoperiod factors (Roberts et al. 1985; Yin, 2008). Both growth chambers were equipped with inflorescent light bulbs with total light intensity of 370 µmol $m^{-2}s^{-1}$ just above the plant canopy.

Three seeds of each accession were planted in 3.8 L pots containing Sunshine mix 4 (Sun Gro, Seba Beach, AB). Within the growth chambers, a total of 27 pots, with three pots for each of the seven transfers plus six control pots for each accession were completely randomized. Starting from one week after crop emergence, the plants were watered every 2 to 3 days based on the growth stage and water use of each accession. Seedlings were thinned to two plants per pot after two weeks and full emergence of the seedlings. Once a week a quick release fertilizer (20 N:20 P_2O_5:20 K_2O) prepared at a concentration of 3 g/l was applied at a rate of 100 ml per pot starting one week after emergence.

Reciprocal transfers between LD and SD were carried out in two time replicates. Control plants were continuously grown at LD and SD. Transfer times for these accessions were customized based on their difference in number of days to flowering under short compared to long days in the previous experiments (Table 5.2). Once plants had been transferred, they were continuously grown in the new chamber under either LD or SD.

Table 5.2. List of chickpea accessions used in the determination of photoperiod-sensitive phase and time of transfer after sowing of each accession from long days to short days and vice versa.

Accessions	Transfer times (days after sowing)						
	1	2	3	4	5	6	7
ICCV 96029	5	10	15	20	25	30	35
FLIP 98-142 C	5	10	15	20	25	30	35
ICC 8855	6	12	18	24	30	36	42
ILC 1687	8	16	24	32	40	48	56
ICC 8621	8	16	24	32	40	48	56
ICC 15294	9	18	27	36	45	54	63
CDC Frontier	10	20	30	40	50	60	70
CDC Corinne	11	22	33	44	55	66	77

5.3 Data Collection and Analysis

5.3.1. Flower bud initiation and full flower opening

First flower bud initiation stage and full open flower appearance (corolla visible) were recorded for each accession. Samples of flower buds were carefully collected from each control and transferred plants in both photoperiod treatments. The stipules were dissected using blades to expose the shoot apex and newly initiated phytomers and a node subtending a leaf primordium, and an auxiliary vegetative or reproductive bud and were evaluated under the microscope. In cases of the death of the first initiated buds, the subsequently formed buds were dissected. Upon seeing some fully developed anthers bounded by a fully developed calyx, the flower bud initiation stages were declared. Days to first flowering for the transfer and control plants in LD and SD were recorded when a fully opened flower appeared on each plant. Days to flower bud initiation and first flowering of the control plants in short and long day photoperiods were used for comparison.

5.3.2 Hinge/non-linear regression to identify hinges, slope coefficients, and Y-intercepts

The hypothetical response of the time from sowing to first flowering for plants transferred from a short day to a long day and from a long day to a short day regime at various time intervals from seeding to first flowering were illustrated in Figure 5.1. Control plants continuously grown under short days are indicated by point A, and those grown under long days are indicated by point E. The intersection point of linear segments AB and CB representing the first hinge for transfer from LD to SD, whereas the intersection point between linear segments of EF and FG represented the first hinge for transfers from SD to LD. Accordingly, the first hinge was calculated as a function of days of transfer from seeding to days to flowering from seeding where the increase or decrease in the slope between the linear segments occur.

The time to flower in the eight chickpea accessions in the successive photoperiod-transfer and control treatments were modelled against the time to transfer after seeding following the procedure of Yin (2008) as illustrated in Figure 5.1. The model of Ying (2008) combines the data from both long-days to short-days and short-days to long-days transfers in a single curve fitting procedure.

Linear segments in Figure 5.1 were further divided into individual linear regression lines that are described in Figure 5.2 with their respective regression function. Each linear segment represents the flowering response of the plants transferred from long to short-days, and from short to long-days illustrating the three developmental phases following a procedure a hinge regression, a simple and effective method that is computationally efficient for locating hinges as described by Breiman (1993). The individual linear segments were exploited to determine the photoperiod sensitive and insensitive phases in chickpea accessions following a procedure described by Wang et al. (1997). The intersection of the two linear equations was deployed to identify the changes in slope and y-intercepts. For plants transferred from long to short days, the intersection point of linear equations Y_{AB} = a3 + xb3 and Y_{BC} = a22 + xb22 was used to identify hinge 1. Similarly, hinge 2 was identified at the intersection point of linear equations Y_{BC} = a22 + xb22 and Y_{CD} = a1 + xb1. For accession transferred from short to long days, hinge 1 was identified at an intersection point of two linear equations: Y_{EF} = a1 + xb1 and Y_{FG} = a2 + xb2 were used to identify hinge 1. Hinge 2 was identified at an intersection point of linear equations: Y_{FG} = a2 + xb2 and Y_{GH}= a3 + xb3. Regression analyses were run for each accession to determine the parameters (a1, b1, a2, b2, first hinge, a22, b22, a3, b3 and second hinge) followed by analyses of variance to determine the difference of the parameters among the accessions.

5.3.3 Identification of photoperiod sensitive and photoperiod insensitive phases

Data on days to flowering for the transfer and control plants were analyzed using the PROC NONLINEAR of SAS version 9.3 (SAS Institute Inc., Cary, NC). Initially, separate data analyses were conducted for each time replicate. The result from separate analysis revealed no significant difference between the results from the two time replicate. In addition, the homogeneity of variance for each time replicates was validated using the Levene's Test. Thus, a combined data analysis was conducted using the average data of the replications in both time replicates for each accession transferred from LD to SD and vice versa and the control plants.

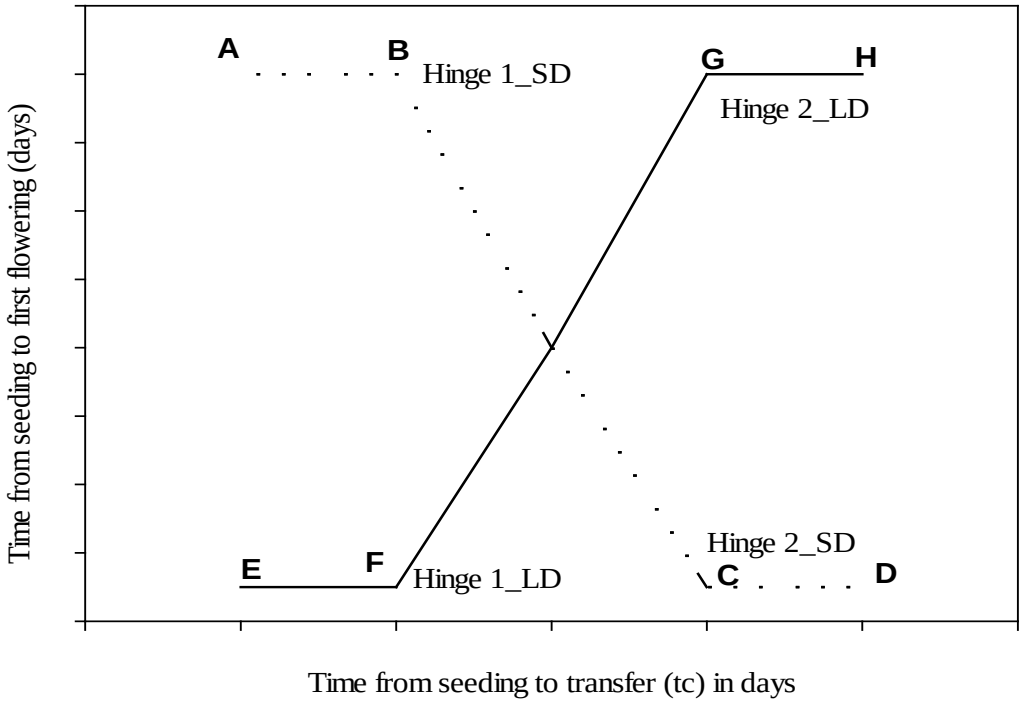

Fig.5.1. Hypothetical representation of the time from sowing to first flowering for plants transferred from a short-day to a long-day (solid line) and from a long-day to a short-day photoperiod regime (dashed line) at specific time points. The first and second hinges for long day (Hinge 1_LD and Hinge 2_LD) were identified as the hinge point at the intersection of line segments EF and GF. Similarly, the first and second hinges for short day (Hinge 1_SD and Hinge 2_SD) were identified as the hinge point at the intersection of line segments AB and CB. The duration of the photoperiod-sensitive inductive phase (durations I_{2s} and I_{2L} in short and long-days, respectively) was included between a photoperiod insensitive pre-inductive and a photoperiod insensitive post-inductive phase. The three sub-phases under the SD conditions are indicated by the linear segments 'AB', 'BC' and 'CD', respectively, and those under the LD conditions are indicated by linear segments 'EF', 'FG' and 'GH', respectively (Ellis at el., 1992; Adams et al., 2003; Yin, 2008).

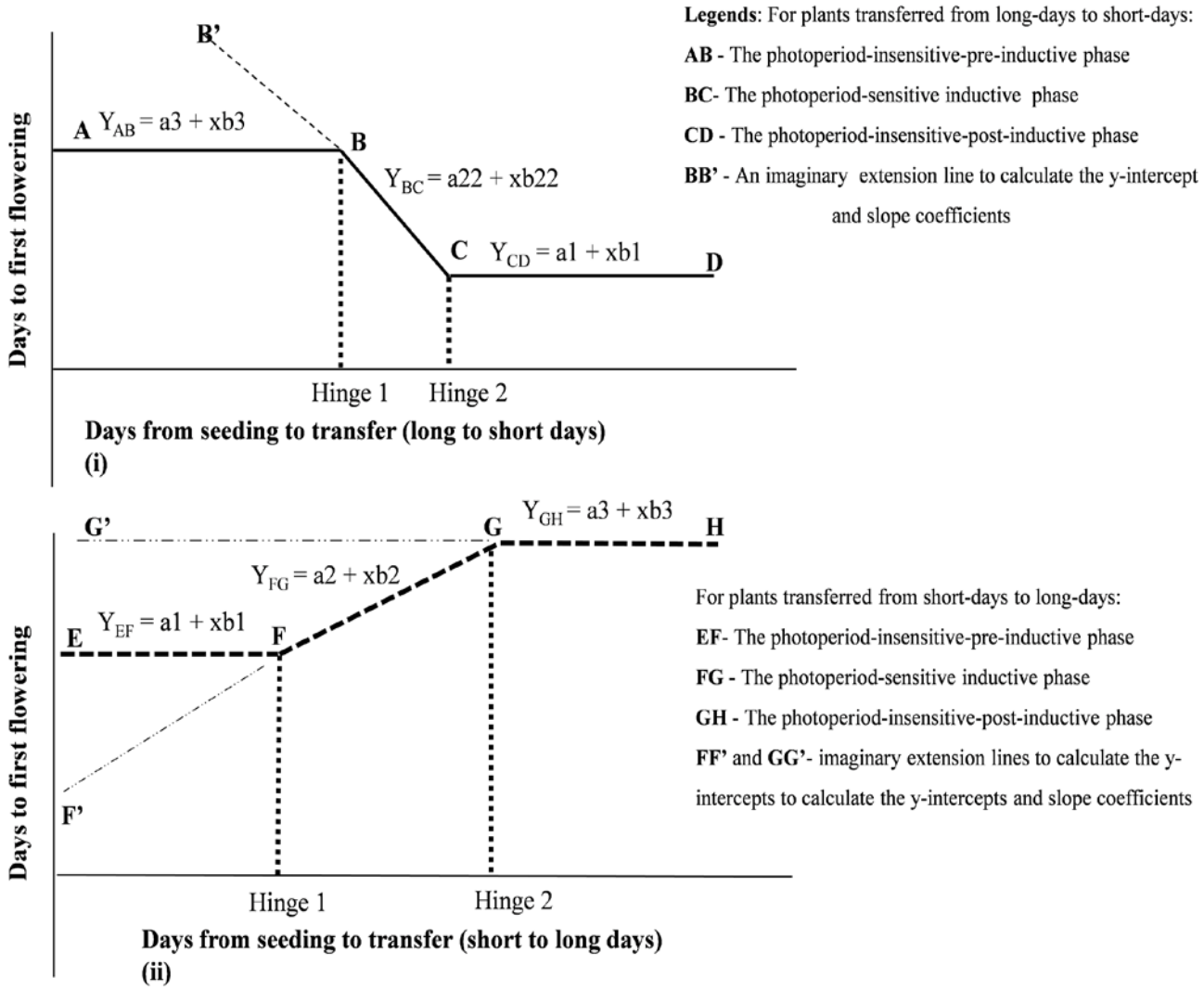

Fig. 5.2 Diagramatic representation for linear regression equations used in the determination of hinge1 and hinge 2 in accessions transferred from (i) long to short-days, and (ii) from short to long-days.

In the analysis, f_L was assigned as the duration from sowing to flowering for the long days and can be written as: $f_L = I_{1L} + I_{2L} + I_{3L}$

where: $I_{1L}$is the first sub-phase, a photoperiod-insensitive pre-inductive phase; I_{2L} is the second sub-phase, a photoperiod-sensitive inductive phase; $I_{3L}$is the third sub-phase, a photoperiod-insensitive post-inductive phase under long day conditions (Yin, 2008).

Similarly f_S was assigned as the duration from sowing to flowering for the short days and the expression can be written as:

$f_S = I_{1S} + I_{2S} + I_{3S}$

where: I_{1S} is the first sub-phase a photoperiod-insensitive pre-inductive phase; I_{2S} is the second sub-phase a photoperiod-sensitive phase; I_{3S} is the third sub-phase, a photoperiod-insensitive post-inductive phase under short day conditions.

5.4 Results

5.4.1. Flower bud initiation and full flower opening

In order to determine the flower bud initiation stage of the plants, flower buds were dissected and visualized under a microscope (X10 magnification) to determine the state of the apex (vegetative or floral) of the main stem, and the branches when they were present (Figure 5.3). The date of floral initiation was defined as the date at which 50 % of the plants had floral apices.

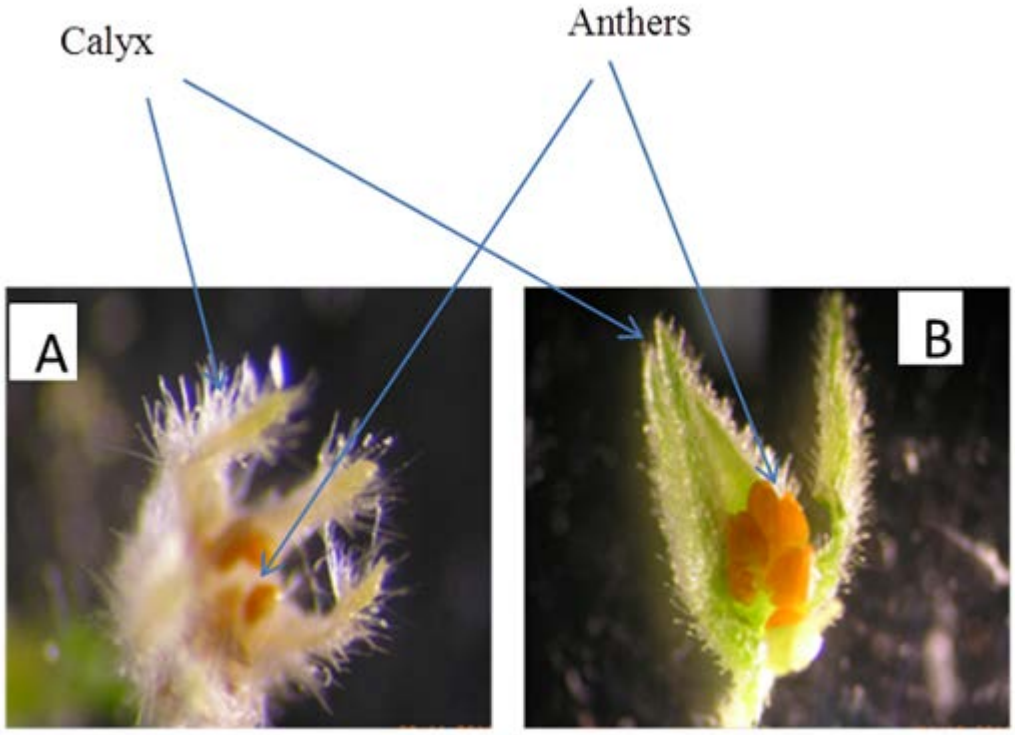

Fig.5.3. Flower bud dissected and visualized under microscope. (X10 magnification, Nikon Eclipse TS100, Japan). Intermediate stage of flower bud initiation stage (A) and fully developed flower bud initiation stage (B).

Control plants grown under the respective photoperiod conditions were used to compare the flowering response of each accession at a given transfer point. The average number of days to flowering was lower for control plants grown continuously under long days compared to those under short-days (Table 5.3). In the photoperiod sensitive accessions, CDC Frontier and CDC Corinne, flowering time was delayed by 45 and 38 days, respectively, in short days compared to long days. Delay in flowering of the 4 accessions with intermediate response to photoperiod ranged from 17 to 42 days in short days compared to long days. In the photoperiod insensitive accessions, ICCV 96029 and FLIP-98-142C, flowering was delayed by 1 and 10 days, respectively, under short days as compared to long days.

Table 5.3. Average number of days from seeding to first flower bud initiation and the number of days to flower under long and short day photoperiod conditions over two time replicates of the experiment.

Accessions	Days from seeding to flower bud initiation		Days from seeding to first flowering	
	LD ± s.d	SD±s.d	LD ± s.d	SD ± s.d
ICCV 96029	19 ± 0.0	19 ± 0.0	28 ± 0.6	29 ± 0.0
FLIP-98-142C	22 ± 0.0	24 ± 0.0	30 ± 0.0	40 ± 0.8
ICC 15294	28 ± 1.0	31 ± 1.0	32 ± 0.5	74 ± 2.9
ILC 1687	28 ± 1.0	31 ± 0.0	41 ± 1.3	66 ± 0.0
ICC 8621	30 ± 0.0	30 ± 12	38 ± 1.3	55 ± 4.1
ICC 8855	29 ± 0.0	30 ± 1.0	38 ± 1.3	68 ± 1.6
CDC Frontier	30 ± 1.0	38 ± 0.0	60 ± 3.3	105 ± 0.9
CDC Corinne	38 ± 1.0	39 ± 1.0	63 ± 2.5	101 ± 1.6

Note: LD = Long days, **SD** = Short days, **s.d** = standard deviation

5.4.2 Slope coefficients, y-intercepts and hinges

The slope coefficients, y-intercepts and the first and second hinges were determined using simultaneous linear regression for the eight chickpea accessions investigated in the reciprocal transfer experiments (Table 5.4).There were significant differences among the chickpea accessions for the second hinge ($P \leq 0.0001$). However, the difference among the accessions for the first hinge and the slope coefficients of the simultaneous equations, were not significant (Table 5.5).

Table 5.4. Means comparison of the first hinge, a1, b1, a2, b2, second hinge and a22, b22, a3 and b3 for eight chickpea accessions evaluated in a reciprocal transfer experiment from long-day (16 hours light) and short-day (10 hours light) photoperiod conditions over two time replicates.

Accessions	First hinge	a1	a2	b1	b2	Second hinge	a22	a3	b22	b3
CDC Frontier	16^{ab}	101^{a}	120^{a}	-0.2^{a}	-1.1^{bbd}	47^{a}	117^{a}	75^{a}	-1.1^{bc}	-0.2^{a}
CDC Corinne	20^{a}	98^{a}	123^{a}	-0.3^{a}	-1.6^{bd}	42^{a}	127^{a}	60^{ab}	-1.7^{c}	0.0^{a}
ICC 15294	20^{a}	76^{b}	115^{ab}	-0.3^{a}	-2.0^{d}	45^{a}	96^{b}	34^{c}	-1.4^{c}	0.0^{a}
ILC 1687	10^{abc}	68^{c}	80^{bc}	0.1^{a}	-1.2^{ddb}	36^{ab}	82^{bc}	36^{bc}	-1.3^{bc}	0.1^{a}
ICC 8621	9^{ac}	59^{d}	67^{dc}	0.0^{a}	-0.9^{bbd}	26^{bc}	76^{c}	40^{bc}	-1.4^{c}	0.0^{a}
FLIP-98-142C	9^{abc}	40^{e}	44^{de}	-0.1^{a}	-0.5^{bc}	26^{bc}	44^{d}	32^{c}	-0.5^{bc}	-0.1^{a}
ICC 8855	3^{bc}	34^{f}	31^{e}	0.0^{a}	1.2^{ab}	19^{c}	11^{f}	72^{a}	3.6^{a}	-0.3^{a}
ICCV 96029	0^{c}	28^{g}	28^{e}	0.0^{a}	0.0^{ab}	1^{d}	29^{e}	27^{c}	-3.0^{d}	0.0^{a}

Note: values with the same letter in the same column are not significantly different; a1, a2, a22 and a3, stand for the Y-intercepts (number of days to flowering); b1, b2, b3, b22, correspond to the slope coefficients for the accessions.

Table 5.5 Analysis of variance for a1, b1, a2, b2, first hinge, a22, b22, a3, b3 and second hinge of the eight chickpea accessions used in the photoperiod sensitive and insensitive phase determination using reciprocal transfers between short-day (10 hours light) and long-day (16 hours light) photoperiod conditions over two time replicates.

Characters	Source of variation			
	df	Sum of Squares	Mean Square	F-Value
a1	7	10995	1571	278^{***}
b1	7	22342	3192	13^{***}
a2	7	0.2	0	1.3^{ns}
b2	7	14	2	6.6^{**}
First hinge	7	805	115	2.9^{ns}
a22	7	30209	4316	100^{***}
b22	7	5136	734	6.6^{**}
a3	7	52	8	57^{***}
b3	7	0.2	0	0.97^{ns}
Second hinge	7	3406	487	15^{***}

Note: a1, a2, a22 and **a3** correspond to y-intercepts in relation to change in number of days to flowering under the respective photoperiods; **b1, b2, b22** and **b3,** stand for slope coefficients in relation to the changes in time from seeding to first flowering against time from seeding to first transfer.

In our analysis the first hinge correspond to a beginning of change in time from seeding to first flowering against time from seeding to transfer. The photoperiod sensitive accessions had the highest values of both the first and second hinge values. The intermediate accessions had intermediate values of both top and bottom hinges. The values of the first and second hinges for the day-neutral accession ICCV 96029 were identified to be 0. The identified hinges facilitated determination of photoperiod sensitive phase in chickpea accessions. The difference between hinge 2 and hinge 1was considered as the photoperiod sensitive phase. Accordingly, in CDC Frontier, a photoperiod sensitive accession, the first hinge and second hinge were 16 and 47 days, respectively. Based on the difference between the first and the second hinges, 31days was considered as the length of the photoperiod sensitive phase of this accession. Similarly, CDC Corinne and ICC 15294 each had the first hinge value of 20 days. The values of the second hinge for these two accessions were 42 and 45 days, respectively. The duration of photoperiod sensitivity of these accessions based on the difference between the second and first hinge were 22 and 25 days, respectively. For other intermediate accessions, the values of the first hinge were 9 to 10 days. The second hinge for these accessions was 19 days. Thus the duration of the photoperiod sensitive phase ranged from15 to 26 days.

5.4.3 Linear regression

Linear regression analysis was conducted for each accession in order to determine the differences in the photoperiod sensitive and photoperiod insensitive phases in the chickpea accessions reciprocally transferred from long to short-days. The slope coefficient values of the accessions were negative for transfers from long to short days (Table 5.6). On the other hand, the slope values of the accessions transferred from short to long days were positive. The slopes for ICCV 96029 transferred either from long to short or short to long days were 0.

Table 5.6. Linear regression for the eight chickpea accessions evaluated in the reciprocal transfer from long days to short days. The y-intercept and slopes presented here are combined over two time replicates.

Experiment	Accessions	y-intercept	slope coefficients	R^2 (%)	CV (%)
Days to flowering of the accessions transferred from long to short days	CDC Frontier	104	-0.65	0.94	5
	CDC Corinne	97	-0.55	0.90	9
	ICC 15294	79	-0.80	0.91	11
	ILC1687	69	-0.65	0.86	10
	ICC 8621	59	-0.45	0.81	9
	ICC 8855	34	-1.00	0.67	8
	FLIP 98-142C	42	-0.40	0.88	5
	ICCV 96029	28	0.00	0.33	2
Days to flowering of the accessions transferred from short to long days	CDC Frontier	58	0.60	0.92	6
	CDC Corinne	53	0.60	0.92	6
	ICC 15294	31	0.70	0.92	8
	ILC1687	39	0.60	0.9	7
	ICC 8621	40	0.40	0.92	5
	ICC 8855	73	0.95	0.85	11
	FLIP 98-142C	29	0.30	0.77	5
	ICCV 96029	28	0.05	0.64	2

LD = Long days, SD = short days; CV = coefficient of variation.

5.4.4 Photoperiod sensitive and photoperiod insensitive phases in chickpea accessions

The reciprocal transfer model fitted the data with R^2 values among the accessions ranging from 0.74 to 0.99 (Table 5.7). All three phases were identified in all the accessions except ICCV 96029. The difference between the flowering time of each accession under the successive transfers were compared with the flowering time of the control treatments.

5.4.4.1 Photoperiod-insensitive pre-inductive phase

In the photoperiod sensitive accessions, a photoperiod-insensitive pre-inductive phase of 15 to 19 days was observed under long days, and 17 to 20 days under short days. These values ranged from 9 to 13 days in the intermediate accessions under long days and from 13 to 18 days under short days. In ICCV 96029, the values of the photoperiod insensitive pre-inductive phase were 22 and 23 days under long and short days, respectively. This value ranges between 18 and 20 days under long and short days, respectively, in FLIP-98-142C.

5.4.4.2 Photoperiod sensitive-inductive phase

The two photoperiod sensitive accessions, CDC Frontier and CDC Corinne, had higher values of photoperiod sensitive inductive phase under short days compared to long days (Table 5.7). In these accessions, the photoperiod sensitive inductive phases under long days ranged from 17 to 38 days, and were 49 to 77 under short days. In the moderately photoperiod-sensitive accessions the photoperiod sensitive inductive phases under long days ranged from 12 to 19 days, and were 25 to 43 under short days.

For ICCV 96029, the values of the photoperiod-sensitive inductive-phase under long-days and short-days were 0.1 and 0.0 respectively. In FLIP-98-142C, another photoperiod-insensitive accession, the overall values of photoperiod sensitive inductive-phase under long and short days were 7 and 15 days, respectively.

Table 5.7. Duration of each of the three developmental phases under long-days (I_{1L}, I_{2L}, and $I_{3L)}$ and under short-days ($I_{1S,}$ I_{2S} and I_{3S}) ± Se from seeding to first flowering in eight chickpea accessions. The values for the three developmental phases were derived from the experiment conducted in two time replicates and the mean values were used in the model to derive the values for each accession.

Accessions	Long-days				Short-days				R^2
	I_{1L}	I_{2L}	I_{3L}	f_L	I_{1S}	I_{2S}	I_{3S}	f_S	
CDC Frontier	15 ± 7	38 ± 9	13 ± 5	66	20 ± 12	77 ± 16	4 ± 10	101	0.98
CDC Corinne	19 ± 3	17 ± 6	20 ± 5	56	17 ± 7	49 ± 14	29 ± 12	95	0.99
ICC 15294	13 ± 9	19 ± 11	5±1.4	35	18 ± 15	43 ± 22	15 ± 18	76	0.97
ILC 1687	12 ± 10	19 ± 14	14 ± 10	45	16 ± 5	40± 9	18 ± 6	74	0.95
ICC 8621	10 ± 1	12 ± 2	20 ± 1	42	13 ± 3	25 ± 4	23 ± 2	61	0.99
ICC 8855	9 ± 5	18 ± 6	11 ± 4	38	14 ± 3	32 ± 5	19± 8	65	0.97
FLIP-98-142C	18 ± 2	7 ± 4	5 ± 2	30	20 ± 3	15 ± 5	6 ± 3	41	0.98
ICCV 96029	22 ± 0.5	0.1 ± 0.9	5 ± 0.9	27	23 ± 0.3	0.0	5 ± 0	28	0.74

I_{1L} = photoperiod insensitive pre-inductive phase
I_{2L} = photoperiod sensitive inductive phase
I_{3L} = photoperiod insensitive post-inductive phase and
f_L = days from seeding to flowering under long days
R^2 = the amount of variation accounted for by the model
I_{1S} = photoperiod insensitive pre-inductive phase
I_{2S} = photoperiod sensitive inductive phase
I_{3S} = photoperiod insensitive post-inductive phase and
f_S = days from seeding to flowering under short days

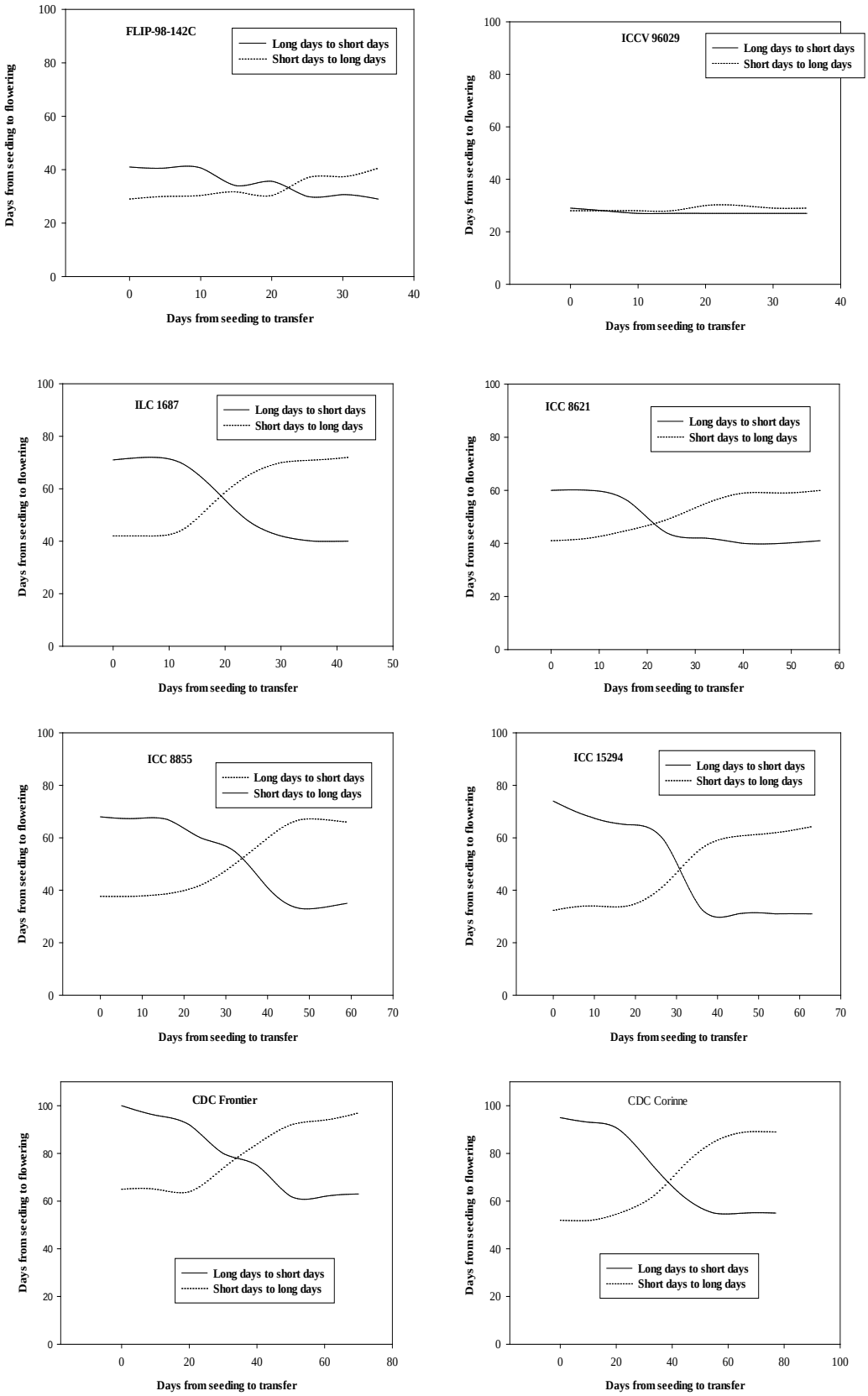

Fig.5.4. The effect of transferring chickpea accessions at varying intervals from long days to short days (solid line) and short-days to long-days (dashed line) on the number of days to first flower opening.

5.4.4.3 Photoperiod-insensitive post-inductive phase

In the highly photoperiod-sensitive accessions, the photoperiod-insensitive post-inductive phases were between 13 and 20 days under long days and 4 to 29 days under short days, respectively. In the intermediate accessions, the values of this phase ranges between 5 and 20 days under long days and 15 to 23 days under short days. The photoperiod insensitive accessions had similar range of photoperiod insensitive post inductive phases of 5 to 6 days in long as well as short days.

5.5 Discussion

When control plants in the respective chambers were compared, the plants under long days flowered earlier than those under short days. Early transfer of plants from either long to short day chambers or *vice versa* had no effect on the flowering response of the plants. Differences in the number of days to flower between short and long day control plants were wider compared to the number of days to flower bud initiation. This indicates that days to full flower opening was delayed by short photoperiod after flower bud initiation. Wallace et al. (1993) reported that time of initiation of flower buds could not be used to differentiate the insensitive and sensitive genotypes in soybean.

Days to flowering of the chickpea accessions transferred from long to short days had negative slope coefficient values ranging from -0.40 to -1.00. On the other hand, the slope of the hinge regression for days to flowering of the accessions transferred from short to long days had positive values ranging from 0.05 to 0.95. The absolute values of the slopes for the photoperiod sensitive and intermediates were higher than the photoperiod insensitive ones. ICCV 96029 specifically had slopes of 0 and 0.05 for transfers from long to short days and short to long days, respectively. Both values are not significantly different from 0, supporting the report that this accession is photoperiod insensitive under a mean temperature of 19˚C combined with either 10 hours or 16 hours photoperiod.

The hinge regression technique was exploited to identify photoperiod sensitive and insensitive phases in the chickpea accessions. The hinge technique was very efficient in differentiating between photoperiod sensitive and insensitive phases in the photoperiod sensitive and intermediate accessions. The advantage of this technique was obvious in the day-neutral aceesiosn ICCV 96029 for which the first and second hinges were 0 and 1, respectively, indicating that significant change in flowering response in this accession was absent, confirming that it is day-neutral under these experimental conditions.

Chickpea has three flowering induction phases: a photoperiod-insensitive pre-inductive phase, a photoperiod-sensitive inductive-phase, and photoperiod-insensitive post inductive phase. An inverse relationship between photoperiod sensitivity phase and photoperiod was identified, i.e. a longer photoperiod sensitive phase was observed under short days, and a shorter photoperiod sensitive phase was observed under long days. Variability in the length of the photoperiod-insensitive pre-inductive phase was observed among the photoperiod sensitive, intermediate, and day-neutral chickpea accessions. A shorter duration of photoperiod insensitive pre-inductive phase was detected compared to the photoperiod sensitive phase in intermediate accessions. During the photoperiod insensitive pre-inductive phase, plants were not responsive to changes in the photoperiod. In many crops, a minimum vegetative period, known as the basic vegetative phase, is required during which there is no response to photoperiod (Vergara and Chang, 1985).

The two high yielding accessions developed and released by the Crop Development Centre, University of Saskatchewan (CDC Frontier and CDC Corinne) (Warkentin et al., 2005) had the longest time to flowering, as well as longer duration photoperiod sensitivity phases under long and short-days. Efforts to develop early flowering cultivars adapted to the short growing season of western Canada could exploit ICCV 96029 and FLIP-98-142C, which have minimal photoperiod sensitive phase. This strategy was also recommended by Kumar and Abbo (2001). Photoperiod insensitivity contributed a significant share for chickpea adaptation to low latitude during early domestication (Siddique et al., 2003; Rubio et al., 2004). Early flowering and maturity in photoperiod insensitive genotypes in bean aided to attain higher harvest index compared to the photoperiod sensitive genotypes (Yourstone et al., 1993).

We concluded that the phenology of chickpea accessions from emergence to first flowering can be divided into three phases: (1) a photoperiod-insensitive pre-inductive

phase, (2) a photoperiod-sensitive inductive phase, and (3) a photoperiod-insensitive post-inductive phase. The duration of the photoperiod-insensitive pre-inductive phase was shorter than that of the photoperiod-sensitive inductive phase in chickpea. Photoperiod sensitivity commenced on different days after emergence in different accessions. The photoperiod-sensitive inductive phase extended beyond flowering bud initiation and full flower opening to the stage of full flower development. We also concluded that flower bud initiation and full flower opening appeared to be sensitive to photoperiod at different times after emergence for different chickpea accessions. Time to flower bud initiation as well as time to full flower opening differentiated photoperiod- insensitive and photoperiod sensitive accessions. In the cool short seasons of Western Canada, chickpea accessions with shorter duration of pre-inductive photoperiod-insensitive and photoperiod-sensitive inductive phases are desirable for adaptation.

6. Mapping QTL for Early Flowering, Photoperiod Sensitivity and Resistance to Ascochyta Blight in a Chickpea Recombinant Inbred Line (CP-RIL-1) Population

Abstract

Ninety-two chickpea Recombinant Inbred Lines (RILs) developed from a cross between ICCV 96029 (desi) and CDC Frontier (kabuli) and the two parents were evaluated for their flowering response under long (16 hours) and short (10 hours) day photoperiods in growth chambers. The difference in the number of days to flower under long vs. short day photoperiods was considered as the photoperiod sensitivity of each line. RILs were also assessed under field conditions for time to flowering, maturity and ascochyta blight reaction and in the greenhouse for ascochyta blight reaction. A wide range of variation was exhibited by the RILs for the time to flowering under short or long day photoperiod, node number of first flower and photoperiod sensitivity under growth chamber conditions, as well as time to flowering and ascochyta blight resistance under field conditions. Ascochyta blight resistance of the RILs under growth chamber and field conditions were positively and significantly ($r = 0.21$, $P \leq 0.05$ to $r = 0.33$, $P \leq 0.001$) correlated. Significant negative correlations were observed among the RILs for days to flowering and ascochyta blight resistance under growth chamber conditions ($r = -0.21$, $P \leq 0.05$ to $r = -0.58$, $P \leq 0.0001$). Photoperiod sensitivity was strongly and positively correlated with the number of days to flowering under short days ($r = 0.91$, $P \leq 0.0001$) and number of nodes to first flowering under short days ($r = 0.80$, $P \leq 0.0001$). Moderate values for broad sense heritability (H^2) for days to flowering under growth chamber conditions (0.86 to 0.87) and in the field (0.45 to 0.78), and for photoperiod sensitivity (0.83) were recorded. Relatively low broad sense heritability was detected for ascochyta blight resistance under greenhouse (0.26 to 0.34) and field (0.14 to 0.68) conditions. A genetic linkage map consisting of eight linkage groups (chromosomes) was developed using 349 marker bins. Seven QTLs were identified

for days to flowering under field and growth chamber conditons. The phenotypic variation explained by these QTLs ranged from 9 to 44%. Two QTLs for days to maturity were identified on chromosomes 3 and 8, explaining 19 and 15% of the phenotypic variation, respectively. Three QTLs were identified for photoperiod sensitivity, one on each of the chromosomes 3, 4 and 5, explaining 7, 11 and 41% of phenotypic variation respectively. Eight QTLs were identified for ascochyta blight resistance across all chromosomes, except chromosome 5. Each QTL explained phenotypic variation ranging from 10 to 19%. In most cases, the genomic regions associated with days to flower were also associated with ascochyta blight resistance and photoperiod sensitivity. The molecular markers associated with these QTLs have potential for use in chickpea breeding.

6.1 Introduction

Chickpea (*Cicer arietinum* L. 2n = 2 x = 16), is one of the most important food legumes in the world next to dry bean (FAOSTAT, 2013). Chickpea has generally been considered as a quantitative long-day plant (Roberts et al., 1980; Roberts et al., 1985; Summerfield and Roberts, 1988). However, recent reports indicated that chickpea is a qualitative long day crop because the rate of development decreased below a critical photoperiod of 11 to 12 hours (Soltani et al., 2004). Due to the qualitative long day nature of the crop, flowering time is significantly prolonged when the crop is exposed to photoperiods of less than 11 to 12 hours. Most chickpea cultivars showed diverse responses in the time to flowering under short days which resulted in low yield because of the increased incidence of malformed flowers and the decreased rate of ripening among late flowering individuals (Roberts et al., 1985). Temperature affects crop development and this development is modified by photoperiod (Takimoto and Hamner, 1964). The phenology of chickpea is controlled by temperature before-flowering, photoperiod and water deficit stress (Roberts et al., 1980).

In India, chickpea is mostly sown in October to November and in Ethiopia from August to January (Van der Maesen, 1972; Bejiga, 1972). In both regions, the growing season is characterized by shortening photoperiod. Small changes in photoperiods in the low-latitude chickpea growing areas of India and Africa suggest that insensitivity alleles at photoperiod response loci had a central role in the successful spread of chickpea into these regions. Such variation might have included alleles at major and minor photoperiod and temperature response loci.

Or et al. (1999) reported the involvement of a major gene affecting the flowering time in chickpea under the Israeli growing conditions. Kumar and van Rheenen (2000) reported that delayed flowering in chickpea is governed by a dominant (*Efl-1*) gene

whereas the recessive (*efl-1*) gene is involved in earlier flowering. Anbessa et al. (2006) reported that the inheritance of time to flowering in chickpea in high latitude, cool-season environments of western Canada followed a two major genes plus polygenes mixed-inheritance model. Quantitative trait loci (QTLs) for flowering time in chickpea have been identified, one each on Linkage Group (LG) 1 and LG 2 (Lichtenzveig et al., 2006) and two on LG 3 (Cho et al., 2002; Cobos et al., 2006). Identification of QTLs on different LGs suggested that there may be several genes for flowering time in chickpea.

Different modes of inheritance of ascochyta blight, caused by *Didymella rabiei*, in chickpea were reported by different authors. A single dominant or a single recessive gene was reported to confer resistance to ascochyta blight (Singh and Reddy, 1993; Tewari and Pandey, 1986; Singh and Reddy, 1996). On the other hand two dominant complementary genes (Dey and Singh, 1993) and at least two recessive genes (Kusmenoglu, 1992) were also reported to confer resistance to ascochyta blight in chickpea. Two to seven QTLs associated with resistance to ascochyta blight at the seedling or adult plant stages were reported in either interspecific or intraspecific populations (Anbessa et al., 2009; Collard et al., 2003; Flandez-Galvez et al., 2003; Udupa and Baum 2003).

Mapping quantitative trait loci in chickpea associated with time to flowering and resistance to ascochyta blight was conducted using a plant population of 120 RILs derived from a cross between the Israeli kabuli cultivar Hadas, which is late to flower, high-yielding, moderately resistant to *D. rabiei*, and an Indian desi accession ICC5810, which is early to flower, low yielding, highly susceptible to the fungus (Lichtenzveig et al., 2006; Bonfil et al., 2006). QTLs on LG2 and LG8 were identified for days to flowering. The genetic correlation between resistance to ascochyta blight and days to first flower was significant and negative indicating that some of the flowering loci are linked to quantitative

loci governing resistance to ascochyta blight. Lichtenzveig et al. (2006) reported that QTLs for resistance to ascochyta blight were also located on LG8 indicating a significant epistatic interaction of the resistance with flowering time.

Seven major genes each in soybean and cowpea and as many as 80 genes in *Arabidopsis* have been reported to affect flowering time and maturity (Koornneef et al., 1998). QTLs associated with flowering time and maturity have been mapped in soybean and a large-effect QTL controlling flowering time, maturity and photoperiod insensitivity were mapped on the same linkage group (Tasma et al., 2001). They also suggested that these traits may be controlled by the same gene or by tightly clustered genes in the same chromosomal region. With QTL mapping the effect of specific loci can be assessed and the interactions between resistance genes, plant development, and the environment can be analyzed. In chickpea, however, only limited information is available on the association of genes for early flowering, maturity, photoperiod insensitivity and ascochyta blight resistance. Therefore, the objectives of this research were to determine the genetic basis of the association between flowering time and resistance to ascochyta blight in chickpea, and to map the chromosome regions that control flowering time, days to maturity, photoperiod insensitivity and resistance to ascochyta blight.

6.2 Materials and Methods

6.2.1 Plant materials

Ninety two chickpea Recombinant Inbred Lines (RILs; population CP-RIL-1) were developed from a cross between ICCV 96029 (desi market class, early maturing, highly susceptible to ascochyta blight) and CDC Frontier (kabuli market class, late maturing, moderately resistant to ascochyta blight) at the CDC, University of Saskatchewan, using the single seed-descent method. ICCV 96029 was developed and released by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, and is considered as one of the earliest flowering chickpea genotypes in the ICRISAT collection (Kumar and Rao, 2001). On average ICCV 96029 flowers 23 days after sowing under indoor conditions. CDC Frontier was released in 2003 by the University of Saskatchewan (Warkentin et al., 2005). This cultivar has a medium-large seed size and high yield potential when grown in the Brown and Dark Brown soil zones of the Canadian prairies. These parents differed by an average of 30 days in days to flowering under the growth chamber conditions.

6.2.2 Phenotyping

6.2.2.1 Evaluation of CP-RIL-1 for response to flowering under short and long days in growth chambers

Indoor experiments to evaluate the CP-RIL-1 population for flowering response under long and short days were conducted in two time replicates under growth chamber conditions. The chambers were adjusted to long days (16/8 hours) or short days (10/14 hours) with a temperature of 22/16°C day and night, respectively, and a light intensity of 370 µmol $m^{-2}s^{-1}$. Each line was planted in 3.8 L pot with 3 pots/line and 2 plants/pot in Sunshine mix # 4 (Sun Gro Horticulture Canada Ltd., Seba Beach, Alberta, Canada). Once

a week plants were fertilized with a fast release fertilizer (20 N: 20 P_2O_5:20 K_2O) at a rate of 3 g/l starting one week after emergence and watered as required.

Days to flower were recorded as the number of days after emergence to when an open flower appeared on a plant. The difference in days to flower between short and long days was used to determine the photoperiod-sensitivity of the plant. Thus, photoperiod sensitivity was measured as the delay in the number of days to flower under short days compared to long days. Node number of first flower appearance was measured by counting the number of nodes on the main stem and/or along any branch to the one subtending the first open flower following a similar procedure as described by Roberts et al. (1985).

Data analysis was conducted using the PROC GLM of SAS 9.3 (SAS Institute Inc., Cary, NC). The Levene's test was conducted for homogeneity of variance for all the measured traits prior to combined analysis from the two time replicates. The experiment was conducted twice using a completely randomized design with three replicates under both conditions in each time replicate. Because there was no significant effect of time replicate, data were pooled over the experimental time replicates. Data were analyzed using a one-way ANOVA model, with lines as main factor for each photoperiod condition. The genotypic variance component was estimated by considering the lines as random factor. Broad sense heritability (H^2) of the measured traits was calculated as the ratio of genetic variance (σ^2_g) to phenotypic variance (σ^2p). Phenotypic variance was estimated as $\sigma^2p = (\sigma^2g + \sigma^2_{er}/nr)$ where σ^2_g, σ^2_{er} are estimates of genotypic and residual error variance, n number of replications and r number of time replicates, respectively. The overall mean of each of the traits in two time replicates under each photoperiod conditions was used for determination of phenotypic correlation coefficients.

6.2.2.2 Evaluation of CP-RIL-1 for flowering response and reaction to ascochyta blight under field conditions

Field experiment to evaluate the flowering response and reaction to ascochyta blight of CP-RIL-1 was conducted at Elrose, Saskatchewan, in 2011 with two replications. In 2012, this experiment was conducted at three locations: Elrose, Moose Jaw, Floral (Saskatchewan Pulse Growers farm) near Saskatoon, Saskatchewan, with three replications at each location. In 2013, the experiment was conducted at two locations: Elrose and Limerick, Saskatchewan, with three replications at each location. In all experiments, a randomized complete block design with microplots of 1 m X 1 m consisting of three rows was used. Planting density was 60 seeds/m^2.

Days to flower were recorded as the number of days after seeding when 50% of the plants within a plot have an open flower. Days to maturity were recorded as the number of days after seeding when 90% of the plants in a plot have turned totan color. Plant height (cm) was measured at full podding stage on the plot basis. In 2012 and 2013, days to maturity were only scored at Elrose. In other locations, Floral and Moose Jaw in 2012 and Limerick in 2013, there was severe ascochyta blight infestation which devastated the plants before they reached maturity. Thus, days to maturity in these locations were not recorded.

Reaction to ascochyta blight was also recorded on a plot basis using a mixed quantitative and qualitative 0-9 score scale, as described by Chongo et al.(2004), where 0 = no symptoms;1 = few very small lesions on leaves and stems, < 2% plant area affected (PAA); 2 = very small lesions, 2-5% PAA; 3 = many small lesions, > 5-10% PAA; 4 = presence of pycnidia, many small lesions, few large lesions, > 10-25% PAA;5 = many large lesions, > 25-50% PAA; 6 = lesions coalescing, > 50-75% PAA; 7 = stem girdling, > 75-90% PAA; 8 = stem breakage, > 90% PAA; 9 = plant dead. The evaluation for reaction

to ascochyta blight was conducted at Elrose in 2011, at Elrose, Moose Jaw and Floral in 2012, and at Elrose and Limerick in 2013.

The years and locations of the field trials were treated as site-years. In Elrose 2011, the experiment was conducted in two replications and data were analysed separately. For each trait measured in the field in 2012 and 2013, homogeneity of variance test were conducted to determine whether combined analysis across site-year was justified. Analysis of variance was done using PROC MIXED of the SAS 9.3 (SAS Institute Inc., Cary, NC). Combined data analyses were conducted for all locations (Elrose, Moose Jaw and Floral farm) in 2012 as well as (Elrose and Limerick) in 2013 for all measured traits. The lines were considered as fixed effects, whereas, location and replicates were considered random effects. Broad sense heritability (H^2) of the characters was estimated as described by Singh et al (1993) as the ratio of genetic variability (σ^2_g) to phenotypic variability ($\sigma^2_g + \sigma^2_{ge} + \sigma^2_e$), where σ^2_g, σ^2_{ge}, σ^2_{er} are estimates of genotypic, genotype by environment interaction and error variance respectively. Phenotypic correlation coefficients were calculated among the traits measured within each site-year. A combined data analysis was conducted for genotype, site-year and their interactions by modelling heterogeneous variances to estimate the overall effect of the environmental variation on phenology and ascochyta blight reactions. To estimate variance components with PROC MIXED, genotype, site-years and their interactions were considered random factors

6.2.2.3 Indoor evaluation of CP-RIL-1 for reaction to ascochyta blight

Four separate evaluations (time replicates) for ascochyta blight reaction of the CP-RIL-1 were conducted in 2011 in the greenhouse complex of the College of Agriculture and Bioresources of the University of Saskatchewan, Canada (Appendix VI). All four experimental time replicates were conducted in the same manner. Two seeds of each RIL and a susceptible check (ICCV 96029) were sown in 10 cm square pots using

Sunshine Mix #4 (Sun Gro Horticulture Canada Ltd., Seba Beach, Alberta, Canada). As a control, two seeds of the susceptible parent ICCV 96029 were placed in each pot to verify that adequate disease pressure had been achieved of the surrounding plants. One week after emergence, the pots were thinned to one RIL and one check plant per pot.

Plants were inoculated with a suspension of the monoconidial *Ascochyta rabiei* isolate Ar-170. This isolate originated from infected chickpea plants collected in 2001 at Swift Current, SK (Dr. Sabine Banniza, personal communication). The inoculum preparation and inoculation process were similar to that described by Tar'an et al. (2007a). Isolate Ar-170 was grown at room temperature under continuous fluorescent light. Inoculum was produced by flooding 7-day-old colonies with sterile distilled water and then agitating the cultures with a sterile rod. The suspensions were filtered through a Mira-cloth layer and adjusted to a final concentration of 2 $x10^5$ conidia/mL using a hemacytometer. Tween 20 (polyoxyethylene sorbitan monolaurate) was added to the suspension as a surfactant at a rate of 1 drop per 100 mL suspension. After inoculation, plants were incubated at 95% relative humidity and 20°C for 48 hours in the dark room. Plants were then transferred to mist benches in the greenhouse equipped with overhead mist nozzles activated for 30 seconds every 90 minutes to keep the plants moist.

Inoculated plants were assessed twice for ascochyta blight severity in each time replicate. The first score (score 1) was recorded 2 weeks after inoculation and the second score (score 2) was recorded 3 weeks after inoculation using a mixed quantitative and qualitative 0-9 score scale, as described by Chongo et al. (2004). Ascochyta blight score 2 was used to estimate the line specific values for ascochyta blight reaction because the two parents showed the most contrasting result at the second evaluation.

Data were analyzed using PROC GLM of SAS 9.3 (SAS Institute Inc., Cary, NC). Analysis was initially conducted for the four experimental time replicates, followed

by a combined analysis. Homogeneity of variance was assumed due to the controlled environmental conditions used. Broad sense heritability (H^2) of ascochyta blight resistance was calculated as previously described. The overall mean of ascochyta blight resistance in four time replicates was used for determination of phenotypic correlation coefficients.

6.2.3 Quantitative trait loci analysis

Linkage map of the CP-RIL-1 was generated using the RAD-seq and Illumina GoldenGate as reported by Deokar et al. (2014). The final map was developed using 349 SNP markers distributed across eight linkage groups. The map spanned 653 cM of the chickpea genome at an average marker density of 1.87 cM. The same genotypic data and linkage map for the CP-RIL-1 were used for the QTL analysis in this study.

QTL mapping was conducted with the composite interval mapping (CIM) implemented in the software package windows QTL Cartographer V2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm: Wang et al., 2007) using this bin map and phenotypic data. The CIM analysis was run using Model 6 with forward and backward stepwise regression, a window size of 10 cM, and a step size of 2 cM. Experiment-wise significance ($p < 0.05$) thresholds for QTL detected were determined with 1000 permutations. The location of a QTL detected was described according to its LOD (Logarithm of odds) peak location and the surrounding region with 95% confidence interval calculated using WinQTLCart. Adjacent QTLs on the same chromosome for the same trait were considered as different QTLs when the support intervals were non-overlapping (if the estimated map position of their peaks fell within 20 cM of each other). The proportion of phenotypic variance (R^2) accounted for by each detected QTL was estimated by a single-factor analysis of variance with the SAS General Linear Model procedure on the individual marker loci closest to the QTL identified by CIM. For QTL nomenclature, a designation begins with ‘‘qtl’’, followed by an abbreviation of the trait

name, the chromosome suffixed with the marker ‘‘-,’’ and finally the serial number of QTL in the chromosome. QTLs with a positive or negative additive effect for a specified trait implies that the increase in the phenotypic value of the trait is contributed by the alleles from ICCV 96029 and CDC Frontier, respectively.

6.3 Results

6.3.1 Flowering response of CP- RIL-1 to short and long day photoperiods under growth chamber conditions

A wide range of variation for days to flower, number of nodes to first flower and photoperiod sensitivity was exhibited by RILs of CP-RIL-1 under growth chamber and field conditions (Table 6.1). Similar broad sense heritability (H^2) estimates under long (0.86) and short day (0.87) photoperiods were obtained. High broad sense heritability for photoperiod sensitivity was also found (0.83). Greater broad sense heritability for node of first flowering was identified under short days (0.75) than under long days (0.37).

Frequency distributions for days to flower of CP-RIL-1 under short and long day photoperiods are presented in Figure 6.1. Days to flower and number of nodes to first flower were significantly greater under short days as compared to long days. On average, number of days to flower and number of nodes to first flower of the CP-RIL increased by 23 days and 6 nodes, respectively, under short days as compared to long days. In the case of ICCV 96029, days to flower and number of nodes to first flower increased by 3 days and 1 node under short days compared to long days. In contrast, for the CDC Frontier, days to flower and number of node to first flower increased by 37 days and 5 nodes, respectively, under short days compared to long days.

Photoperiod sensitivity of the two parents was 4 and 37 days for ICCV 96029 and CDC Frontier, respectively (Figure 6.2). ICCV 96029 was considered as day-neutral (photoperiod-insensitive) because of the small difference in the number of days to flower under short days compared to long days.

Table 6.1. Analysis of variance for days to flower (Dtf) under long and short days, number of node to first flower (Node) under long and short-days and photoperiod sensitivity (PS) for CP-RIL-1 (92 RILs and the parents, CDC Frontier and ICCV 96029) evaluated in growth chamber conditions over two time replicates.

Photoperiod		Short days		Long days		PS
Characters		Dtf	Node	Dtf	Node	
Source	df	F-Value		F- Value		
Genotype	93	21***	9.87***	18.9***	2.76***	15.43***
Error	443					
Total	536					
Var. comp.						
σ^2_g		334.9	19.8	30.4	1.91	242
σ^2_{er}		49.8	6.7	5.1	3.3	50.3
H^2		0.87	0.75	0.86	0.37	0.83
cv		14.2	14.4	10.2	14.9	23.1

σ^2_g and σ^2_{er} stand for genotypic and error variance, respectively. PS = photoperiod sensitivity, derived as delayed number of days to flower under short days as compared to long days; *** indicates significant difference at $P \leq 0.0001$.

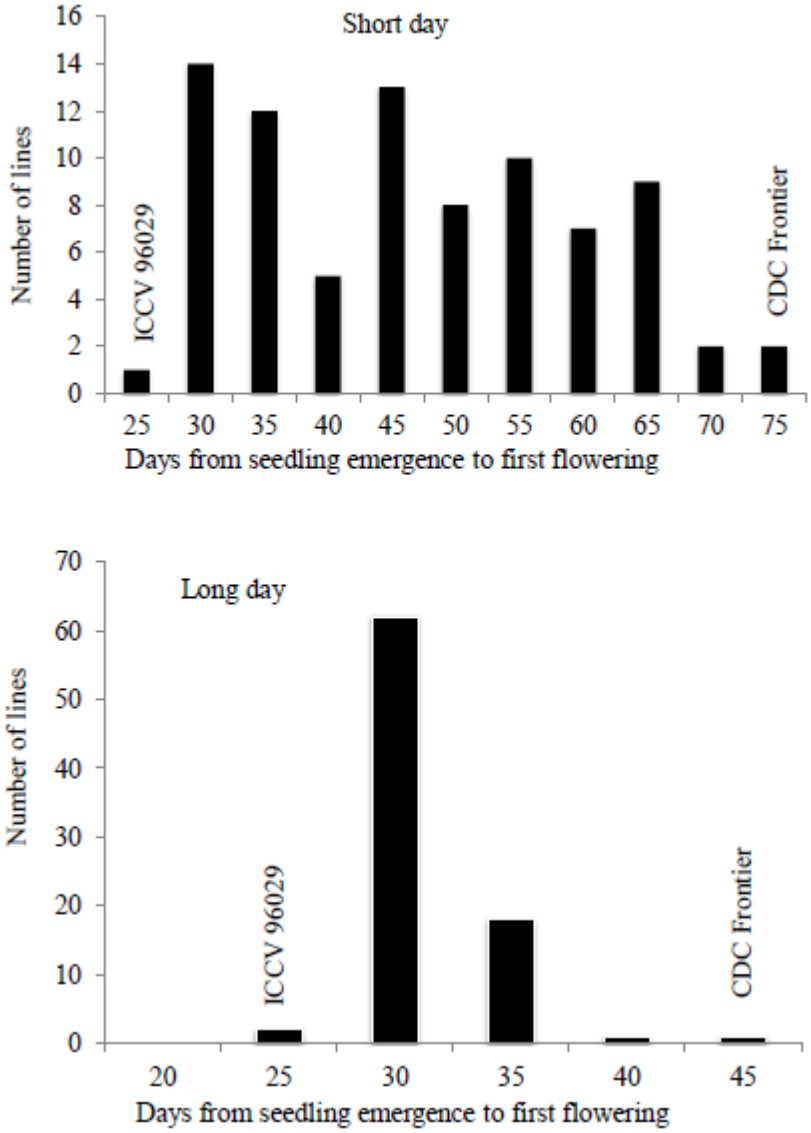

Figure 6.1 Frequency distributions for days to flower for 92 RILs and the parents (CDC Frontier and ICCV 96029) of chickpea population CP-RIL-1 evaluated under short-day (10 /14 hours day /night) and long-day (16/8 hours day /night) photoperiods averaged over two time replicates; the temperature of the growth chambers was adjusted to 22/16°C (days and night respectively).

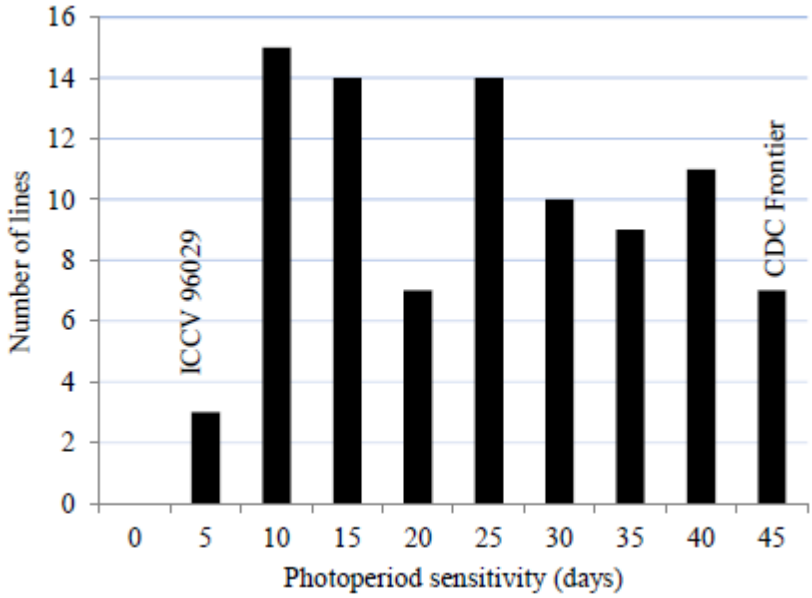

Fig. 6.2. Frequency distribution for photoperiod sensitivity for the CP-RIL-1 (92 RILs and the two parents, CDC Frontier and ICCV 96029). Photoperiod sensitivity was calculated based on the delay in number of days to flowering under short days (10/14 hours days and night) compared with long days (16/8 hours day and night). The data presented is averaged over two time replicates. Mean temperature was 22/16°C (day and night, respectively).

6.3.2 Flowering response and reaction to ascochyta blight of CP- RIL-1 under field conditions

Analysis of variance indicated significant difference among the RILs for days to flowering, plant height and ascochyta blight resistance across three locations in 2012 and 2013. However, there were no significant differences among the RILs for ascochyta blight resistance at Elrose in 2011 (Table 6.2). There were significant effects of genotype, environment, and their interaction for days to flowering in both years. The interaction of genotype and environment was not significant for plant height in 2012, and for ascochyta blight score 2 in 2012 and in 2013. Low to moderate broad sense heritability for days to flowering (0.45), plant height (0.48) and ascochyta blight (0.14) were recorded in 2011. Similar broad sense heritabilty for days to flower (0.71 to 0.78) and plant height (0.75 to 0.78) were detected in 2012 and 2013. The mean and range of the population for the measured traits were intermediate between ICCV 96029 and CDC Frontier (Table 6.3).

Table 6.2 Analysis of variance for days to flowering (Dtf) ascochyta blight scores 1 and 2 (Ab1 and Ab2), Plant height (Plht), days to maturity (Dtm) for CP-RIL-1 evaluated at Elrose in 2011, Elrose, Moose jaw and Floral farm in 2012 and Elrose and Limerick in 2013.

Year	Location	Sources	Dtf	Plht	Ab1	Ab2	Dtm
2011	Elrose	df					
	G	93	2.79^{***}	2.9^{***}	0.95^{ns}		
	Er	88					
	Total	181					
	Var. Comp.						
	σ^2_{er}		2.9	11.7	1.4		
	σ^2_{g}		2.4	10.81	0.2		
	H^2		0.5	0.48	0.1		
	cv		3.2	11.8	24		
2012	Elrose, Moose Jaw and Floral						
	G	93	12.3^{***}	12.2^{***}	5.3^{***}	4.9^{***}	
	E	2	3647^{***}	1099^{***}	1254^{***}	858^{***}	
	G x E	186	2.7^{***}	1.15^{ns}	1.5^{***}	1.2^{ns}	
	Er	564					
	Total	845					
	Var. Comp.						
	σ^2_{er}						
	σ^2_{g}		28	88	1.3	1.4	
	σ^2_{e}		7.4	23	0.9	1.1	
	σ^2_{ge}		4	1.2	0.2	0.1	
	H^2		0.7	0.8	0.6	0.6	
	cv		5	10	21	18	
2013	Elrose and Limerick						
	G	93	10.4^{***}	10^{***}	7.1^{***}	7.4^{***}	2.1^{***}
	E	1	19.6^{***}		92.2^{***}	578.4^{***}	
	G x E	93	2.0^{***}		1.4^{*}	1.3^{ns}	
	Er	93					
	Total	186					
	Var. Comp.						
	σ^2_{er}			12.19			11
	σ^2_{g}		6.13	36.96	0.6	0.74	3.86
	σ^2_{e}		0.3	-	0.2	1.47	-
	σ^2_{ge}		1.5	-	0.08	0.06	-
	H^2		0.78	0.75	0.68	0.33	0.26
	cv		4.5	8	14	12	19

G, E, GxE and Er are genotype, environment, genotype by environment interaction and error; σ^2_{g}, σ^2_{ge}, σ^2_{e}, σ^2_{er} are estimates of genotypic, genotype by environment interaction, environment and error variance respectively. CV = coefficient of variation, *,**,*** indicates significant difference at $P \leq 0.05$, 0.01and 0.001, ns = non-significant, respectively.

Table 6.3 Mean and range values of CP-RIL-1 and parents for characters assessed under field and/or growth chamber conditions.

Characters	Locations	Population		ICCV 96029		CDC Frontier	
		mean	range	mean	range	mean	range
Days to flowering	Growth chamber						
	Long days	22 ± 5	22-53	19 ± 2	16-21	47 ± 2	44-50
	Short days	34 ± 16	23-80	21 ± 2	16-25	84 ± 3	81-88
Nodes to flowering	Long days	12 ± 1	9-16	10 ± 2	9-11	16 ± 1	14-17
	Short days	15 ± 4	12-25	11 ± 3	9-16	20 ± 2	17-24
Photoperiod sensitivity		23 ± 13	11-54	4 ± 2	0-7	37 ± 4	31-44
Days to flowering	Elrose (2011)	53 ± 0	48-59	50 ± 1	50-51	59 ± 1	58-60
	Elrose (2012)	53 ± 6	42-66	42 ± 0	42-42	66 ± 0	64-68
	Elrose (2013)	56 ± 4	29-63	48 ± 1	47-49	66 ± 1	65-66
	Elrose (Mean)	54 ± 1	29-66	47 ±3	42-51	64 ± 3	58-66
	Moose Jaw (2012)	63 ± 2	56-66	52 ± 0	51-61	66 ± 0	66-66
	SPG (2012)	44 ± 5	39-63	39 ± 2	38-40	63 ± 2	56-67
	Limerick (2013)	32 ± 6	25-39	25 ± 0	25-25	39 ± 0	39-39
Plant height (cm)	Elrose (2011)	30 ± 0	19-45	25 ± 1	24-25	34 ± 1	33-35
	Elrose (2012)	50 ± 8	42-69	40 ± 3	36-42	56 ± 5	52-62
	Moose Jaw (2012)	38 ± 6	22-55	31 ± 2	28-34	47 ± 3	44-51
	SPG (2012)	58 ± 8	36-79	40 ± 0	40-40	66 ± 5	62-74
	Elrose (2013)	46 ± 7	29-63	40 ± 2	38-42	54 ± 5	49-59
	Elrose 2012	114 ± 3	107-123	112 ± 3	109 -115	116 ± 0	116-116
Days to maturity	Elrose (2013)	97 ± 4	88-113	92 ± 0	92-92	97 ± 1	96-98

ANOVA of combined data for DTF, AB1, AB2, PLHT, and DTM was conducted for genotype, site-year, and their interactions indicating significant ($P \leq 0.001$) effect of site-year and interaction of genotype by site-year, but not genotype (Table 6.4). From 24 to 92% of the variation observed in DTF, PLHT and DTM could be explained through site-years indicating the importance of the environmental effect. Moderate heritability (0.22 to 0.66) was detected for the traits assessed (Table 6.5), which was lower than the heritability values estimated for separate site years.

Table 6.4 Analysis of variance for DTF, AB1, AB2, PLHT and DTM in the CP-RIL-1 evaluated over five site-years (Elrose-2012, Floral-2012, Moose Jaw-2012, Elrose-2013 and Limerick-2013) in 2012-2013.

Variables	DTF	AB1	AB2	PLHT	DTM
	F-Value				
Genotype	0.1^{ns}	0^{ns}	0.01^{ns}	0.21^{ns}	1.2^{ns}
Site-year	629.54^{***}	31.62^{***}	20.2^{***}	113.65^{***}	712.19^{***}
Genotype x Site-year	2.61^{***}	1.74^{***}	1.55^{***}	1.75^{***}	1.41^{*}

*,*** indicates significant difference at $P \leq 0.05$ and 0.001 respectively; ns = non-significant.

Table 6.5 Estimates of variance components (% explained by the components explained as proportion of the total variance) and heritability of the DTF, AB1, AB2, PLHT and DTM the 92 RILs of the CDC Frontier and ICCV 96029 population grown at five site-years.

Variances	DTF	AB1	AB2	PLHT	DTM
Genotype	2.4 (3.6)	0.2 (7)	0.2 (7.4)	15 (12.4)	0.7 (0.5)
Site-year	48.6 (74.7)	1.1 (36.7)	0.5 (24.4)	67.6 (55.9)	137 (91.9)
Genotype x Site-year	4.9 (7.6)	0.3 (9.9)	0.2 (9.9)	9.1 (7.5)	1.9 (1.3)
Error	9.1 (14)	1.4 (46.3)	1.3 (58.4)	29.3 (24.2)	10.7 (7.2)
Total	65	3	2.2	121	148.9
H^2	0.46	0.37	0.40	0.66	0.22

Chickpea is seeded during the month of May in Saskatchewan, with flowering beginning in late June to early July depending on environmental and agronomic conditions. Daily temperature and precipitation data, collected by Environment Canada at standard meteorological stations, was obtained at http://weather.gc.ca (Table 6.6). The site-years differed substantially in total precipitation during the growing seasons, i.e., substantially greater total precipitation was recorded at the 2012 site-years compared to the 2011 and 2013 site-years, while mean growing season temperature was similar at all site-years. Amount and timing of precipitation affects the establishment, growth and flowering of plant communities (Inouye et al., 2003), and has a major impact on disease development. Greater total precipitation during 2012 as compared to 2011 and 2013 increased vegetative plant growth leading to later flowering, taller plants, later maturity, greater ascochyta blight ratings, and the significant effect of site-year in the combined analysis (Table 6.4). The effect of genotype was significantly manifested in the genotype by site-year interaction (Table 6.5).

Table 6.6. Monthly total precipitation and monthly mean air temperature at Elrose (2011-2013), Moose Jaw (2012) Floral farm (Saskatoon) and Limerick (2013) during May-August.

Location		Elrose			Floral Farm	Moose Jaw	Limerick
Year		2011	2012	2013	2012	2012	2013
Mean Temperature (˚C)	May	10.5	10.1	13.2	13.0	10.5	12.7
	June	15.2	16.0	16.0	15.5	16.7	15.7
	July	17.5	19.6	17.2	17.4	20.1	17.9
	August	17.0	17.8	18.5	18.9	17.9	19.3
	Overall average	15.1	15.9	16.2	16.2	16.3	16.4
Total Precipitation (mm)	May	20.6	100.2	15.9	108.0	116.1	60.2
	June	52.8	150.6	105.6	121.1	45.4	56.7
	July	74.2	47.0	37.6	80.9	78.2	33.5
	August	29.2	22.6	20.4	48.5	43.2	18.0
	Total	176.8	320.4	179.5	358.5	282.9	168.4

6.3.3 Reaction of the CP-RIL-1 to ascochyta blight under growth chamber conditions

CP-RIL-1 was phenotyped for reaction to ascochyta blight isolate of Ar-170 using a 0 - 9 scale. Ascochyta blight score 2 was used for data analysis because the two parents showed the most contrasting result at the second evaluation. Initially data from single time replicates were analysed separately before combined analysis was conducted. Combined analyses were conduced for time replicates 1 and 2 as well as for time replicates 3 and 4. Significant differences were detected among the RILs in their reaction to ascochyta blight scores in all time replicates (Table 6.7). Moderate broad sense heritability of 0.18 and 0.34 were estimated for ascochyta blight score 2 respectively in time replicates 1 and 2, and time replicates 3 and 4. ICCV 96029 on average had a score of 7.0, whereas CDC Frontier had a score of 4.0. The ascochyta blight scores of the CP-RIL-1 population were averaged over the four replicates. The mean ascochyta blight scores of the CP-RIL-1 population in four replicates ranged from 4.7 to 6.6, and mean scores of the RILs ranged from 2 = very small lesions, (2-5% PAA) to 9 = plant dead, in all the four time replicates (Table 6.8).

Table 6.7 Analysis of variance for ascochyta blight in the CP-RIL-1 evaluated in greenhouse conditions in 2011. In each time replicate, ascochyta blight score 2 was conducted 3 weeks after inoculation.

Time replicates	Sources	df	F-value
1 and 2			
	G	93	1.87^{***}
	Er	222	
	Total	315	
	Var. Comp.		
	σ^2_{er}		2.04
	σ^2_g		0.44
	H^2		0.18
	Cv		24.48
3 and 4	G	93	3.06^{***}
	Er	528	
	Total	621	
	Var. Comp.		
	σ^2_{er}		1.28
	σ^2_g		0.66
	H^2		0.34
	cv		17.13

Note: df = degree of freedom; G and Er are genotype and error, respectively; σ^2_g and σ^2_{er} are estimates of genotypic and error variances; cv = coefficient of variation, *** indicates significant difference at $P \leq 0.001$.

Table 6.8. Summary of the mean and range of ascochyta blight reaction for the CP-RIL-1 and parents evaluated in greenhouse conditions in 2011 and under field conditions in 2011-2013. Data presented for the greenhouse conditions were based on the mean values of score 2 (three weeks from inoculation) over replications in each time replicate. Data for the field conditions were based on the year of the experiment at each location.

Environment	Population		ICCV 96029		CDC Frontier	
	mean	range	mean	range	mean	range
Greenhouse replicate -1	4.7 ± 0.9	2.0-8.0	7.0 ± 1.0	5.0-7.0	4.0 ± 0.0	4.0-4.0
Greenhouse replicate -2	6.0 ± 0.8	3.8-8.9	7.5 ± 1.0	7.0-8.0	5.0 ± 1.0	4.0-6.0
Greenhouse replicate -3	5.6 ± 0.8	3.9-9.0	7.0 ± 1.0	6.0-9.0	4.2 ± 1.0	3.0-6.0
Greenhouse replicate -4	6.6 ± 1.0	4.8-8.8	7.8 ± 0.5	7.0-8.0	4.5 ± 1.0	4.0-5.0
Greenhouse mean	5.7 ± 0.9	3.6-8.7	7.3 ± 0.9	6.3-7.8	4.4±0.8	3.8-5.3
Elrose (2011)	4.0 ± 1.0	2.0-8.0	5.0 ± 1.0	5.0-6.0	4.0 ± 1.0	3.0-4.0
Elrose (2012)	6.0 ± 0.8	4.0-9.0	7.8 ± 1.1	6.5-9.0	4.3 ± 1.0	4.0 -5.0
Elrose (2013)	5.0 ± 0.9	3.3-8.7	7.8 ± 0.7	7.0-8.7	3.8 ± 0.2	3.7-4.0
Elrose mean	5.0 ± 1.0	2.0-9.0	7.0 ± 1.3	5.0-9.0	4.0 ± 0.2	3.0-5.0
Moose Jaw (2012)	7.0 ± 1.0	4.0-9.0	7.5 ± 1.0	7.2-7.8	4.7 ± 1.0	4.0-5.5
Floral (SPG farm) (2012)	7.0 ± 1.0	5.0-9.0	8.0 ± 1.0	7.0- 9.0	3.8 ± 1.0	3.5-4.0
Limerick (2013)	6.0 ± 1.0	2.5-9.0	7.5 ± 1.0	7.0-9.0	3.0 ± 1.0	3.0-4.0

6.3.4 Correlation of traits under field and indoor conditions

6.3.4.1 Days to flower under growth chamber and field conditions

Days to flowering under growth chamber conditions were averaged over two time replicates and the field data at Elrose were averaged over three years (2011-2013) for correlation analysis. For the other three locations (Floral-2012, Moose Jaw-2012 and Limerick-2013), the data were averaged over replications in a single year. Significant and positive correlations were observed between days to flower and ascochyta blight resistance under growth chamber conditions (Table 6.9). Days to flower under long day photoperiod in the growth chamber was positively and significantly correlated with days to flower under field conditions: Elrose ($r = 0.21$, $P \leq 0.05$), Limerick ($r = 0.52$, $P \leq 0.0001$), Floral ($r = 0.61$, $P \leq 0.0001$) and Moose Jaw ($r = 0.35$, $P \leq 0.05$). Similarly, days to flower under short day photoperiod was positively and significantly correlated with days to flower under field conditions: Elrose ($r = 0.21$, $P \leq 0.05$), Limerick ($r = 0.54$, $P \leq 0.0001$), Floral ($r = 0.55$, $P \leq 0.0001$), Moose Jaw ($r = 0.50$, $P \leq 0.0001$).

6.3.4.2 Ascochyta blight reaction indoor and under field conditions

Similar to days to flowering, the greenhouse data for ascochyta blight resistance used for correlation analysis were averaged over four time replicates and the field data at Elrose were averaged over three years (2011-2013). For the other locations, the data were averaged over replications in a single year. Ascochyta blight reactions of the CP-RIL-1under growth chamber and field conditions were positively and significantly correlated. The coefficients of correlation of ascochyta reaction under growth chamber and each location are following: Elrose ($r = 0.32$, $P \leq 0.001$), Limerick ($r = 0.21$, $P \leq 0.05$), Floral ($r = 0.33$, $P \leq 0.01$), Moose Jaw ($r = 0.33$, $P \leq 0.001$) (Table 6.6).

Table 6.9 Pearson correlation coefficients for days to flower, days to maturity and ascochyta blight resistance evaluated under field conditions (Elrose in 2011-2013, Moose Jaw and Floral in 2012 and Limerick in 2013), ascochyta blight under greenhouse conditions, days to flower and number of node to first flowering under long days, days to flower and numbed of node of first flower appearance under short days and photoperiod sensitivity of the CP-RIL-1.

Characters	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
DTF_Elr (1)	0.19^{ns}	0.29**	0.29**	0.10^{ns}	0.17^{ns}	0.21*	0.25*	0.25*	0.22*	0.21*	0.21*	0.18^{ns}	-0.02 ^{ns}	-0.09 ^{ns}	-0.07 ^{ns}	-0.22*	-0.21*
DTF_Limk (2)		0.72***	0.47***	0.25*	0.28**	0.42***	0.48***	0.49***	0.34***	0.54***	0.52***	0.44***	-0.21*	-0.40***	-0.32**	-0.58***	-0.34***
DTF_Flor (3)			0.57***	0.21*	0.39***	0.46***	0.53***	0.52***	0.44***	0.55***	0.61***	0.44***	-0.16^{ns}	-0.45***	-0.30**	-0.58***	-0.41***
DTF_Msj (4)				0.31**	0.28**	0.39***	0.36***	0.51***	0.27*	0.50***	0.35***	0.46***	-0.34**	-0.50***	-0.24*	-0.44***	-0.43***
DTM_Elr (5)					0.07^{ns}	0.19^{ns}	0.07^{ns}	0.21*	-0.07^{ns}	0.25*	0.02^{ns}	0.22*	0.01^{ns}	-0.36***	-0.05^{ns}	-0.31**	-0.11^{ns}
PLHT_Elr (6)						0.57***	0.58***	0.21*	0.31**	0.16^{ns}	0.22*	0.12^{ns}	-0.09^{ns}	-0.14^{ns}	-0.13^{ns}	-0.17^{ns}	-0.27**
PLHT_Flor (7)							0.75***	0.36***	0.33**	0.31**	0.35***	0.28**	-0.17^{ns}	-0.36***	-0.15^{ns}	-0.45***	-0.40***
PLHT_Msj (8)								0.27**	0.34**	0.23*	0.31**	0.18^{ns}	-0.04^{ns}	-0.23*	-0.19^{ns}	-0.33**	-0.40***
Node_SD (9)									0.24*	0.82***	0.31**	0.80***	-0.15^{ns}	-0.30**	-0.19^{ns}	-0.47***	-0.24*
Node_LD (10)										0.17^{ns}	0.57***	0.08^{ns}	-0.1^{ns}	-0.13^{ns}	-0.37**	-0.27**	-0.29**
DTF_SD (11)											0.39***	0.91***	-0.15^{ns}	-0.40***	-0.29**	-0.47***	-0.21*
DTF_LD (12)												0.24*	-0.08^{ns}	-0.26*	-0.2^{ns}	-0.41***	-0.27**
PS (13)													-0.11^{ns}	-0.38***	-0.28**	-0.41***	-0.16^{ns}
AB_Gh (14)														0.32**	0.21*	0.33**	0.33**
AB_Elr (15)															0.31**	0.62***	0.6***
AB_Limk (16)																0.29**	0.43***
AB_Flor (17)																	0.48***

Note: DTF_Elr (1) = Days to flower at Elrose
DTF_Limk (2) = Days to flower at Limerick
DTF_Flor (3) = Days to flower at Floral
DTF_Msj (4) = Days to flower at Moose Jaw
DMAT_Elr (5) = Days to maturity at Elrose
PLHT_Elr (6) = Plant height at Elrose
PLHT_Flor (7) = Plant height at Floral
PLHT_Msj (8) = Plant height at Moose Jaw
Nod_SD (9) = Node to first flowering under short days
Nod_LD (10) = Node to first flower under long days
DTF_SD (11) = Days to flower under short days
DTF_LD (12) = Days to flower under long days
PS (13) = Photoperiod sensitivity
AB_Gh (14) = Ascochyta blight in greenhouse
AB_Elr (15) = Ascochyta blight at Elrose
AB_Limk (16) = Ascochyta blight at Limerick
AB_Flor (17) = Ascochyta blight at Floral
AB_Msj (18) = Ascochyta blight at Moose Jaw

,** and * indicated significance at $P \leq 0.05$, 0.01 and 0.0001, respectively; ns = non-significant

6.3.4.3 Ascochyta blight and days to flower

There was significant negative correlation between days to flower under field condition and ascochyta blight resistance in the field conditions ($r = -0.32$ to -0.58, $P \leq 0.001$) (Table 6.9). Days to maturity (recorded at Elrose, only) and ascochyta blight resistance were significantly ($r = -0.36$, $P \leq 0.001$) and negatively correlated.

6.3.4.4 Photoperiod sensitivity

Photoperiod sensitivity and days to flower under field and growth chamber conditions, plant height, and number of node of first flower appearance were positively correlated (Table 6.9). The strongest correlation was between photoperiod sensitivity and number of node to first flower under short days ($r = 0.80$, $P \leq 0.0001$), and days to flower under short days ($r = 0.91$, $P \leq 0.0001$). Photoperiod sensitivity was correlated with days to flowering ($r = 0.44$ to 0.46, $P \leq 0.0001$), days to maturity at Elrose ($r = 0.22$, $P \leq 0.05$), and plant height at Floral ($r = 0.28$, $P \leq 0.001$). Photoperiod sensitivity was ($r = 0.24$, $P \leq 0.05$) and positively correlated with days to flower under long days in growth chambers.

6.3.4.5 Days to flower, plant height and node of first flowering

Plant height was positively correlated with days to flowering under field conditions of Floral ($r = 0.36$ to 0.46, $P \leq 0.0001$). Plant height in the field conditions and node number of first flowering under short day and long day photoperiod in growth chambers were positively and significantly associated.

6.3.5 QTL for days to flower and days to maturity in CP-RIL-1

The number and locations of the QTLs identified for days to flower, days to maturity, number of node to first flower, photoperiod sensitivity and ascochyta blight resistance for CP-RIL-1 are indicated in Table 6.10 and Figure 6.3.

QTLs for days to flowering (*qtlDtf-3.1*, *qtlDtf-4.1*, *qtlDtf-4.2*, *qtlDtf-4.3*, *qtlDtf-4.4*, *qtlDtf-5.1*and *qtlDtf-8.1)* were identified on chromosomes 3, 4, 5 and 8. The QTL on chromosome 4 (*qtlDtf-4.1)* was consistent across locations and years Floral-2012, Elrose-2012 and Moose Jaw, Elrose-2013 and Limerick-2013, as well as under long and short day photoperiods in growth chambers. Another QTL for days to flowering (*qtlDtf-5.1*) was identified on chromosome 5, accounting for 44% of the total phenotypic variation. A consistent QTL for days to flowering across locations and years (Elrose-2011, Elrose-2012 and Moose Jaw-2012) was identified on chromosome 8 explained on average 17% of the phenotypic variation. Similarly, QTL for days to maturity were identified on chromosomes 3 (*qtlDtm-3.1*) and 8 (*qtlDtm-8.1*) explaining 19 and 15% of the total phenotypic variation, respectively.

6.3.6. QTL for photoperiod sensitivity

Photoperiod sensitivity was derived as delay in days to flower under short days as compared to long days. When photoperiod sensitivity was used as an independent variable, three QTLs were identified on chromosomes 3 (*qtlPs-3.1),* 4 (*qtlPs-4.1)* and 5 (*qtlPs-5.1*). These QTLs explainined phenotypic variations of 7 to 41%. The QTL on chromosome 5 explained the highest proportion of phenotypic variation. The QTL on chromosome 5 was located between 42.92-70.8 cM with the additive effect of -8.55 (LOD = 18.81).

Table. 6.10. Quantitative trait loci (QTL) detected for days to flower (Dtf), days to maturity (Dtm), Plant height (Plht), photoperiod sensitivity (Ps), number of node to first flowering (Node) and ascochyta blight resistance (Ab) in CP-RIL-1 evaluated under growth chamber and field conditions in 2011-2013.

Characters	QTL	Chromosome	Closest markers	Position (cM)	Interval (cM)	LOD	PVE (%)	Additive
Ab	qtlAb-1.1	1	CAV1SC21.1P1495114	19.8	18.8-21.1	3.3	13	0.6
Plht	qtlPlht-1.1	1	CAV1SC28.1P1164750	32.7	32.7 -53.0	7.9	26	3.3
Ab	qtlAb-2.1	2	scaffold905p1129574	41.4	41.4-47.2	3.9	14	0.38
Ab	qtlAb-3.1	3	CAV1SC548.1P43520	8.6	8.6-23.5	4.6	15	0.4
Dtf	qtlDtf-3.1	3	CAV1SC48.1P396061	7.4	7.43-23.11	5.3	9	-4.1
Dtm	qtlDtm-3.1	3	CAV1SC48.1P396061	7.4	7.43-18.54	5.4	19	-1.4
Node	qtlNod-3.1	3	scaffold1777p70396	5.8	5.75-25.52	5.8	11	-1.1
Plht	qtlPlht-3.1	3	CAV1SC390.1P214265	63.8	65.8 -75.1	4.9	25	-2.5
Ps	qtlPs-3.1	3	CAV1SC48.1P396061	7.9	7.9-23.52	4.7	7	-3.5
Ab	qtlAb-4.1	4	scaffold405p948196	34	15.5-36.2	4.8	17	0.47
Dtf	qtlDtf-4.1	4	scaffold2005p25023	51.9	51.6-51.9	3.1	11	-0.9
Dtf	qtlDtf-4.2	4	scaffold360p479554	15.5	15.48-26	5.7	10	-4.4
Dtf	qtlDtf-4.3	4	CAV1SC2.1P566504	31.4	31.42-35.61	3.8	13	-1.3
Dtf	qtlDtf-4.4	4	scaffold34p1977386	3	3.01-14.71	5.7	14	-0.5
Node	qtlNode-4.1	4	scaffold360p644415	22.4	22.35-26	4.2	10	-1
Node	qtlNod-4.2	4	CAV1SC2.1P3082421	57.9	42.92-70.8	9.4	29	-1.8
Ps	qtlPS-4.1	4	scaffold360p644415	20.4	16-26	5.8	11	-4.3
Dtf	qtlDtf-5.1	5	CAV1SC1.1p4940145	61	42.55-70.8	18	44	-9.6
Ps	qtlPS-5.1	5	CAV1SC1.1p4940145	55.9	42.92-70.8	19	41	-8.6
Ab	qtlAb-6.1	6	CAV1sc445.1p92883	13.2	26.9-52.7	5.5	19	0.41
Ab	qtlAb-7.1	7	CAV1SC102.1P548827	45	45.0-57.7	3.2	10	0.29
Ab	qtlAb-8.1	8	CAV1SC679.1P39451	72	72 -75.8	3.6	12	0.26
Ab	qtlAb-8.2	8	scaffold1567p981540	1.6	1.6-14.4	5.4	16	0.41
Ab	qtlAb-8.3	8	scaffold21p63604	53.8	53.8-54.3	3.2	9	0.32
Dtf	qtlDtf-8.1	8	scaffold937p67148	53.8	53.77-62.33	4.3	17	-1.3
Dtm	qtlDtm-8.1	8	scaffold1567p981540	0	0.01-4.61	3.3	15	-1.1
Node	qtlNod-8.1	8	scaffold1439p220499	45.8	45.77-69.62	9.3	32	-0.8

PVE= Phenotypic variation explained by the QTL (in %); Positive and negative additive effects indicated increased effects contributed by CDC Frontier and ICCV 96029, respectively.

1
0.0 CAV1SC15.1P1077795
2.3 CAV1SC15.1P901225
5.3 scaffold101p655539
7.7 scaffold101p1733815
10.7 scaffold101p2251236
13.1 scaffold101p2764466
14.2 scaffold101p2786728
14.8 scaffold101p3119612
15.4 CAV1SC19.1P1750067
16.6 CAV1SC19.1P1017184
18.3 CAV1SC19.1P497841
18.8 CAV1SC19.1P354162
20.5 CAV1SC21.1P1096693
21.0 CAV1SC21.1P1495114
23.4 scaffold101p4768558
24.6 scaffold101p5390644
25.2 scaffold101p5509488
28.2 scaffold101p6149516
28.8 scaffold101p6154460
30.5 CAV1SC80.1P96583
31.6 CAV1SC388.1P158402
32.8 scaffold550p16814
33.4 scaffold550p1752067
33.9 scaffold550p1752071
34.5 CAV1SC151.1P36873
35.1 CAV1SC908.1P31832
36.9 CAV1SC239.1P93424
37.5 scaffold187p798253
38.2 scaffold32p20836
38.7 CAV1SC47.1P173767
43.1 CAV1SC326.1P75884
43.7 scaffold447p288448
51.6 scaffold3294p700295
52.2 CAV1SC28.1P1164750
53.5 scaffold3294p593695
64.3 CAV1SC10.1P1068069
64.8 CAV1SC10.1P756169
65.4 CAV1SC10.1P533315
75.5 CAV1SC23.1P951533
qtlAb-1.1
qtlPlht-1.1
2
0.0 CAV1SC40.1P395341
1.8 scaffold521p216256
8.5 scaffold2283p1470498
9.6 scaffold2283p1137579
10.2 scaffold2283p1134921
11.4 scaffold2283p942461
13.9 scaffold2283p237532
14.5 CAV1SC141.1P89095
15.6 CAV1SC140.1P330267
18.6 CAV1SC174.1P495336
21.5 CAV1SC143.1P73663
22.1 scaffold1471p212654
25.4 scaffold1176p188168
27.6 scaffold2307p728748
28.3 CAV1SC584.1P119047
29.0 scaffold500p576492
33.6 scaffold191p466392
34.3 scaffold2193p113525
35.1 scaffold256p72724
35.9 scaffold657p314477
36.6 scaffold270p658556
37.9 scaffold1318p467858
39.1 scaffold887p538591
39.8 scaffold58p890246
40.4 scaffold58p286681
42.4 scaffold621p326093
43.0 scaffold1204p2760
43.7 scaffold2159p69275
44.9 scaffold905p1129574
45.5 scaffold905p693958
46.0 CAV1SC57.1P1051835
47.2 scaffold905p636294
47.8 scaffold1180p283355
48.4 scaffold78p640871
51.3 CAV1SC689.1P195825
51.9 CAV1SC817.1P81915
53.7 scaffold183p1539190
54.3 scaffold183p890167
54.9 CAV1SC246.1P169941
55.4 CAV1SC246.1P121732
63.7 scaffold137p10777
67.9 CAV1SC112.1P378362
69.1 CAV1SC112.1P613679
69.6 CAV1SC103.1P136659
70.2 scaffold449p722985
70.8 CAV1SC103.1P100830
71.4 scaffold449p830576
qtlAb-2.1
3
0.0 scaffold308p1446195
3.6 scaffold1101p3466
4.3 CAV1SC179.1P475444
5.0 scaffold291p297065
5.7 scaffold662p108135
6.4 scaffold718p841535
7.9 scaffold1213p167814
8.6 scaffold227p149371
9.3 scaffold342p303880
10.0 scaffold165p76711
10.6 scaffold228p196897
11.3 CAV1SC48.1P396061
12.0 scaffold1777p70396
13.5 scaffold364p86929
15.8 CAV1SC548.1P43520
17.9 scaffold1313p59187
18.5 scaffold498p29571
20.5 scaffold714p220477
21.1 CAV1SC71.1P237291
23.5 scaffold28p2999987
28.6 scaffold28p1702903
33.2 scaffold687p513070
35.7 scaffold1871p14410
36.9 scaffold1871p875930
40.0 scaffold1871p1641253
40.6 scaffold1871p1719965
41.2 scaffold1871p2358126
41.8 CAV1SC24.1P381212
47.0 CAV1SC68.1P614741
48.7 CAV1SC68.1P136177
49.2 scaffold1208p665588
49.8 scaffold1208p582465
52.8 CAV1SC111.1P585303
53.3 CAV1SC111.1P557263
55.0 CAV1SC111.1P284754
60.5 CAV1SC3.1P2460923
62.2 CAV1SC3.1P1595446
63.3 CAV1SC3.1P1477115
63.8 CAV1SC3.1P1297039
72.2 CAV1SC26.1P645183
75.1 CAV1SC152.1P79343
qtlAb-3.1
qtlPS.3.1
qtlDH-3.1
qtlNod-3.1
qtlDtm-3.1
qtlPlht-3.1
4
0.0 CAV1SC9.1P693069
3.5 CAV1SC9.1P1232713
4.1 CAV1SC9.1P1582445
4.6 scaffold34p1977386
6.3 CAV1SC9.1P2145342
6.9 scaffold1978p148211
8.0 scaffold1978p872430
8.6 scaffold1978p1122196
9.1 CAV1SC25.1P1927917
10.2 CAV1SC25.1P1095606
10.8 scaffold1758p2675534
11.4 scaffold1758p3567468
12.5 scaffold1758p2146922
13.7 scaffold1758p1888469
15.5 scaffold1758p1006151
16.1 CAV1SC58.1P571352
16.6 scaffold360p479554
17.2 CAV1SC58.1P346798
17.8 CAV1SC2.1P5035513
18.3 scaffold360p644415
24.9 CAV1SC2.1P3633116
26.0 CAV1SC2.1P3082421
26.6 CAV1SC2.1P2945721
27.1 scaffold405p948196
27.7 CAV1SC2.1P2866837
31.4 scaffold405p2153718
32.0 CAV1SC2.1P1872075
32.6 CAV1SC2.1P1435160
34.4 scaffold405p3186244
35.0 CAV1SC2.1P566504
35.6 CAV1SC2.1P1129668
36.2 scaffold405p3450200
39.2 scaffold77p2859561
39.8 scaffold77p2742838
42.2 scaffold77p1818751
42.8 scaffold77p1747620
43.9 CAV1SC317.1P224893
45.1 scaffold77p31132
45.7 scaffold1326p154972
46.4 scaffold1326p37841
47.6 CAV1SC129.1P443461
48.1 CAV1SC77.1P175059
48.7 CAV1SC129.1P265821
51.2 scaffold1351p194481
51.8 scaffold2005p25023
52.5 scaffold320p2751278
53.6 scaffold320p2686095
54.2 scaffold320p2599156
56.1 scaffold3794p57002
56.7 scaffold3258p54397
59.8 scaffold75p697
60.4 CAV1SC39.1P119450
61.0 scaffold27p2264111
61.6 CAV1SC39.1P72391
64.7 CAV1SC39.1P935727
67.6 CAV1SC242.1P528294
68.1 CAV1SC242.1P311297
71.6 CAV1SC69.1P500580
73.4 scaffold1534p1404651
73.9 scaffold1534p1421244
74.5 CAV1SC69.1P28720
75.1 CAV1SC69.1P3429
76.2 CAV1SC132.1P261467
83.1 CAV1SC36.1P711069
86.0 CAV1SC183.1P40364
qtlAb-4.1
qtlDtf-4.2
qtlPS.4
qtlNod-4.1
qtlDtf-4.1
qtlNod-4.2

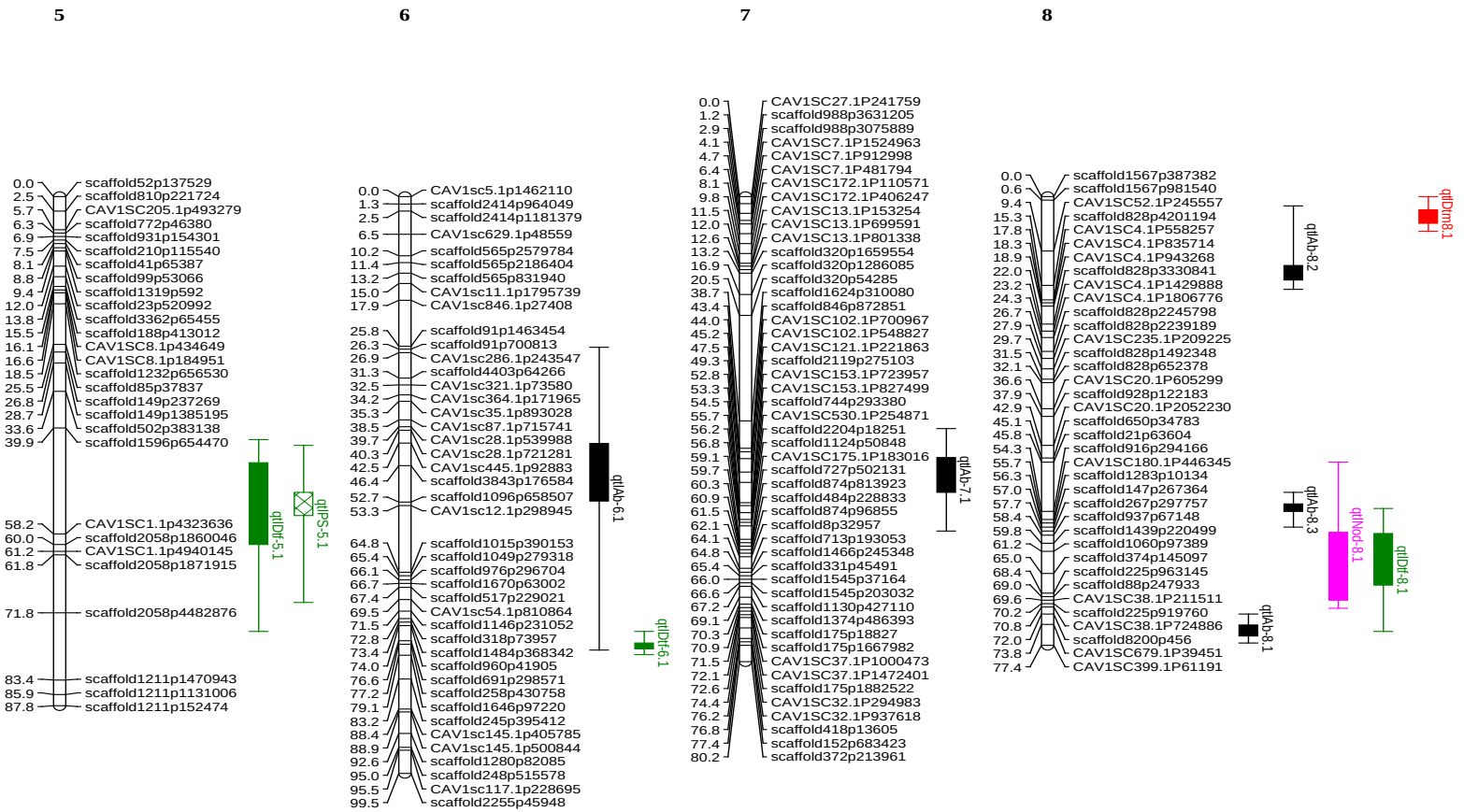

Figure 6.3 QTLs identified for days to flower, days to maturity, plant height and ascochyta blight resistance under field (Elrose, Floral, Moose Jaw and Limerick in 2011-2013 seasons) and growth chamber conditions as well as photoperiod sensitivity and number of node to first flower under long and short day photoperiod treatments on 8 (1- 8) chromosomes in CP-RIL-1 derived from a cross between CDC Frontier and ICCV 96029.

6.3.7 QTL for plant height and node of first flowering

QTLs for plant height were identified on chromosomes (*qtlPlht-1.1*) 1 and 3 (*qtlPlht-3.1*) acounting for 26 and 25% of the total phenotypic variation, respectively. Four QTLs for node of first flower were detected in CP-RIL-1 evaluated under growth chamber conditions. Three of the 4 QTLs for days to flowering were identified for days to flowering under short day conditions while 1 QTL was identified for days to flowering under long day conditions. The QTL identified for node of first flowering under short days was located on chromosome 3 (*qtlNode-3.1*) at a position of 5.75-25.52 cM (PVE = 11%), two QTLs on chromosome 4 (*qtlNode-4.1*) at 22.35-26 cM (PVE=10%) and 42.92-70.8 cM (PVE = 29 %). The only QTL for node of first flower under long day was identified on chromosome 8 (*qtlNode-8.1*) at a position of 45.77-69.62 cM with an additive effect of -0.76 (LOD = 9.29) explaining 32% of phenotypic variation.

6.3.8 Quantitative trait loci for ascochyta blight resistance

QTLs for ascochyta blight were identified on all chromosomes except chromosome 5. On chromosome 1, QTL for ascochyta blight resistance under greenhouse conditions (*qtlAb-1.1*) was identified explaining 13% of the total phenotypic variation. This QTL was consistent across time replicates 1 and 3 in the greenhouse. A QTL for ascochyta blight resistance under field conditions was identified on chromosome 2 (*qtlAb-2.1*) explaining 14% of the total phenotypic variation. Chromosome 3 and 4 contained QTL (*qtlAb-3.1* and *qtlAb-4.1*) with 15-17% of the total phenotypic variation by each QTL. These QTLs were consistent across field and greenhouse conditions (1-4 time replicates) and over years: Elrose-2011, Elrose-2012, Floral-2012, Moose Jaw-2012, and Elrose-2013). One QTL was identified on chromosome 6 (*qtlAb-6.1*) at the interval of 13.22-62.3cM, contributing a total phenotypic variation of 19%. This QTL is consistent across locations and years, including Limerick-2013, Elrose-2011, Moose Jaw-2012, and Elrose-2012. One

QTL was identified on chromosome 7 (*qtlAb-7.1*) at an interval 45.0-57.7 cM contributing 10% of a total phenotypic variation. Three QTLs for ascochyta blight resistance (*qtlAb-8.1, qtlAb-8.2* and *qtlAb-8.3*) were identified on chromosome 8, explaining a total phenotypic variation of 9 to 16%. Q*tlAb-8.2* was consistent across years and locations: Limerick-2013, Floral-2012, Elrose-2013 and Elrose-2012.

6.3.9 Co-localization of QTLs and traits correlation

Chromosome 3 contained a cluster of QTLs for days to flowering, days to maturity, photoperiod sensitivity and number of node of first flowering in the region spanning 7.43- 25.52 cM. The co-localization of these traits was confirmed by the significant and positive correlation among these traits. Cluster of QTLs for days to flowering, photoperiod sensitivity and node number of first flowering under short days was identified on chromosome 4 at an interval of 15.48-70.8cM. The co-localization of QTL for node number of first flowering and photoperiod sensitivity was supported with the strong and positive correlation between the traits ($r = 0.80$, $P \leq 0.0001$). Days to flowering under short days and photoperiod sensitivity were highly correlated ($r = 0.91$, $P \leq 0.0001$).

6.4 Discussion

Evaluation of the RILs for a series of measurements under controlled and field conditions indicated considerable variation among the lines for their reaction to ascochyta blight, in the number of days to flowering and photoperiod sensitivity. Moderate to high values for broad sense heritability (H^2) for days to flowering (0.45 to 0.87) and photoperiod sensitivity (0.83) were detected. Rehman et al. (2011) reported a similar result for days to flowering under drought condition ($H^2 = 0.71$), however, wider ranges of broad sense heritability for days to flowering were reported by Anbessa et al. (2006) ($H^2 = 0.65$ to 0.97) and Varshney et al. (2014a) ($H^2 = 0.21$ to 0.96). A low broad sense heritability of 0.36 was reported by Cobos et al. (2007) and of 0.38 to 0.54 by Lichtenzveig et al. (2006). The moderate values for broad sense heritability for time to flowering suggest that chickpea could be improved for this trait in western Canada. Varshney et al. (2014) reported that selection for traits with high broad sense heritability such as time to flower will be effective in breeding.

A significant and negative correlation was identified between days to flowering and ascochyta blight resistance under field and growth chamber conditions. Similarly ascochyta blight resistance and days to maturity were significantly and negatively correlated. The negative correlation indicates that early flowering and maturing RILs display high susceptibility to ascochyta blight. This negative correlation could be due to linkage of some of the flowering loci with QTL governing resistance to ascochyta blight. Our finding is in agreement with several previous reports, which indicated a significant negative genetic correlation between resistance to ascochyta blight and days to first flower in chickpea (Lichtenzveig et al., 2002, 2006; Aryamanesh et al., 2010). Kumar and Abbo (2001) also reported that the late flowering lines were ascochyta blight tolerant and the earlier flowering lines were ascochyta blight susceptible.

A significant effect of environment and interaction of environment by genotype were detected in this study for days to flowering, days to maturity, ascochyta blight resistance and plant height. This variation was mainly attributed to the substantial difference in total precipitation during the growing seasons. Complex traits are highly influenced by the interaction of genotype and the environment and this interaction is a common and important source of variation (Purcell, 2002; Long et al., 2007). Flowering phenology is dynamic and is related to both temperature and precipitation (Lesica and Kittelson, 2010; Swemmer et al., 2007) as was the case in the research reported in this thesis.

Photoperiod sensitivity and days to flowering under short days, photoperiod sensitivity and node number of first flowering under short days, days to flowering under short days and node number of first flowering under short days had coefficient of correlations ranging from 0.80 to 0.91. Correlation coefficients with absolute values greater than 0.71 are useful to predict more than 50% of the variance of one trait by the other in classical breeding (Skinner, et al., 1999). The correlation of these traits also supported the common QTLs shared by these traits on chromosome 3 at an interval of 5.75 to 25.52 cM. There is limited previous information on photoperiod sensitivity in chickpea. In the present study, we have phenotyped the RILs under controlled photoperiod to detect the photoperiod sensitivity of the lines. Three QTLs were detected on chromosomes 3, 4 and 5. The markers associated with the QTLs for photoperiod sensitivity on chromosome 3 could be useful for candidate gene analysis and marker assisted selection in chickpea breeding, since QTLs for other traits including ascochyta blight, days to flowering, days to maturity and node number of first flowering were located in this interval.

Four QTLs (qtlDtf-4.1, qtlDtf-4.2, qtlDtf-4.3 and qtlDtf-4.4) for time to flowering were identified on chromosome 4. Cobos et al. (2007) identified a QTL for days to flowering (QTL_{DF1}) in LG4, closely linked to STMS GAA47 in chickpea intraspecific

recombinant inbred line derived from a kabuli by desi cross under field and growth chamber conditions. It is not conclusive whether the QTLs identified were the same or different from the previously reported QTLs. Two additional QTLs (qtlNode-4.1and qtlNod-4.2) were identified at an interval of 22.35 to 26 and 42.92 to 70.8 cM on this chromosome.

Important QTLs for days to flowering under short days (qtldtf-5.1) and photoperiod sensitivity (qtlPs-5.1) were identified on chromosome 5 at an interval of 42.92-70.8 cM, explaining 44 and 41% of the total phenotypic variation, respectively. The high coefficient of correlation between these traits and high broad sense of heritability estimates suggest that time to flowering and photoperiod sensitivity are affected by the same genes.

The recombinant inbred line population used in this study was derived from a cross between CDC Frontier, a kabuli cultivar developed and released for cold temperate growing environments of western Canada, and ICCV 96029, a desi chickpea developed and released for production in the semi-arid environments of India, thus, different alleles responsible for flowering are expected to be present. A QTL was identified for days to flowering on LG3 between the markers Ts57 and Ta127 developed by Cho et al. (2002) using RILs derived from a cross between ICCV 2 and JG 62. Jamalabadi et al. (2013) identified a QTL for days to flowering on linkage group 3 using RILs derived from ILC 3279 and ICCV 2 flanked between the markers Ta117 and CaSTMS22 spanning a total distance of 1.3 cM. The identified QTL for days to flowering on LG3 in a chromosomal region of 7.43-23.11 cM, could be the same as the QTL reported by Jamalabadi et al. (2013) since we used ICCV 96029, which was developed from a cross between ICCV 2 and ICCV 93929.

In this study, QTLs for ascochyta blight resistance were identified on all chromosomes except chromosome 5. Previous studies identified QTLs for ascochyta blight resistance on several linkage groups. QTLs for ascochyta blight resistance were reported on

LGs1 and 3 by Flandez-Galvez et al. (2003), on LG2 and 4 by Udupa and Baum (2003). QTLs on LG3, LG4 and LG6 were identified for ascohyta blight resistance in an F_2 population derived from ICCV 96029 x CDC Frontier (Anbessa et al., 2009). In this study, QTLs for days to flowering and ascochyta blight resistances were found to be clustered in the same region on chromosome 8 at interval of 53.88-62.33 cM. A QTL for node of first flowering (qtlNod-8.1) was indentified in the overlapping interval of 45.77 to 69.62 cM on this chromosome explaining the highest (32%) phenotypic variation.

A QTL for resistance to ascochyta blight and days to flowering on linkage group 8 was previously reported by Lichtenzveig et al. (2006). SeveralQTLs for time to flowering and ascochyta blight resistance were identified within the same genomic region in the current study and this may have given rise to the negative correlation between these traits. QTLs for time to flowering and ascochyta blight resistance were also identified at different chromosomes, which can be exploited for simultaneous improvement of the traits in chickpea.

One of the main objectives of chickpea breeding in western Canada is to develop early maturing and ascochyta blight resistance cultivars. Previous studies indicated that days to flowering and maturity in chickpea were positively and significantly correlated (Anbessa et al. 2007*a*; Varshney et al., 2014*a*). Early flowering and early maturing lines also tend to be highly succeptible to ascochyta blight. The negative correlation between earliness and ascochyta blight resistance is undesirable for breeders. Further analysis is needed to confirm whether the correlation is due to tight linkage or pleiotropy. In case of linakage between two allels, availability of these alles on different arms of chromosome may aid plant breeders to bring favourable combinations of genes together. Thus, identification of another flowering time gene, not linked to the locus for ascochyta resistance, can be used for earliness (Gaur et al., 2007; 2014). Pyramiding genes from different genetic backgrounds may aid in

developing cultivars with earliness and ascochyta blight resistant. Desirable traits such as early flowering (Anbessa et al., 2006); ascochyta blight resistance in chickpea (Tar'an et al., 2007*b* ; Anbessa et al., 2009); time to flowering and plant height in lentil (Tullu et al., 2008) could be improved by gene pyramiding.

In conclusion, this study identified QTLs related to early flowering, plant height and ascochyta blight resistance in chickpea recombinant inbred lines derived from ICCV 96029 and CDC Frontier. For the first time in chickpea, QTLs for photoperiod sensitivity, number of node of first flowering, were reported. The QTLs identified in this study in relation to flowering time, days to maturity, ascochyta blight resistance and photoperiod sensitivity are useful for marker assisted approaches and could be exploited in chickpea breeding programs.

7. General Discussion

The first step towards maximizing crop yield by agronomic management or plant breeding is to ensure that the phenology of the crop is well matched to the resources and constraints of the production environment. Because chickpea is grown throughout a wide geographical range and in various cropping systems, it is exposed to several biotic and abiotic stresses during the growing season (Roberts et al., 1985; Singh 1997). These stresses include terminal drought in the arid and semi-arid environments and end of season frost in western Canada. Terminal drought is the major yield limiting factor in the Mediterranean and tropical growing environments. On the other hand, in areas characterized by short-growing-season such as western Canada, the excessive vegetative growth due to a moderate rainfall during the late summer exposes the crop to killing frost before maturity (Gan et al. 2009). The longer crop duration also prolongs the exposure of the crop to biotic stresses such as ascochyta blight (Bonfil et al. 2006). Thus, time to flowering is an essential component of chickpea adaptation in the semi-arid, short growing season of western Canada.

In Canada late maturity and susceptibility of the crop to ascochyta blight is a major bottleneck of production. Thus, development of early maturing and ascochyta blight resistant cultivars are desired for the sustainable production of the crop. Flowering time in chickpea is a function of genotype, temperature and photoperiod (Roberts et al.,1985). Early flowering contributes to decreased days to maturity in chickpea (Anbessa et al., 2006). In wheat and rice, selection for photoperiod insensitive genotypes has been a major achievement in wider adaptation of the crops (Marshall et al., 1989; Worland et al., 1998). Seletion for early flowering would improve the timely maturity of chickpea in Western Canada ultimately improving yield and quality (Anbessa et al., 2006). Thus, understanding of the genetic control of these individual factors and their interaction would fill the gap in understanding earliness, which is desired in chickpea for western Canada.

Wider adaptation in wheat was achieved as a result of the development of less photoperiod sensitive genotypes (Kamran et al., 2014). A combination of photoperiod insensitivity and temperature insensitivity is required to ensure early flowering in chickpea (Or, et al., 1999). However, there is limited information on the genetic diversity of the crop for their response to photoperiod. Identification of a desirable and genetically diverse germplasm source is a crucial step to select parental materials for crop improvement programmes in grain legumes. Utilization of the available genetic diversity in chickpea germplasm would enhance the development of cultivars adapted to the short growing environments of western Canada.

There were significant differences in the number of days to flowering and node of the first flower in the chickpea accession evaluated under short and long day photoperiods. This indicated the existence of diversity in chickpea accessions for their response to flowering under different photoperiod. The level of photoperiod sensitivity measured as a difference in the number of days to flowering under short days as compared to longer days indicated those genotypes originated from around the tropics and lower latitudes environments were less sensitive to photoperiod. On the other hand those genotypes that originated from and were adapted to the higher latitudes and more temperate climates like western Canada were highly sensitive to changes in photoperiod.

Photoperiod sensitivity was significantly and positively correlated with the number of days to flowering and node of first flower under short days. In addition to flowering, photoperiod also affects several reproductive traits such as pod setting in plant (Zhang et al., 2001). Thus, it is essential to understand the response of chickpea genotypes for their response to changes in photoperiod in order to select and develop adapted genotypes and also for proper application of cultural practices.

Overall earlier flowering of the accessions was observed under longer photoperiods and higher temperature regimes. All chickpea accessions used in the present study flowered at a faster rate under long day photoperiod compared with short day photoperiod. Similarly, earlier flowering was observed under higher temperature regimes compared to lower temperature regimes. The overall mean days to flowering of the accessions were 37, 46 and 64 days under 24/16 ºC, 20/12 ºC, and 16/8 ºC, diurnal temperatures regimes respectively.

The interaction effect of accessions, temperature and photoperiod on the flowering responses were significant. Contrast comparison confirmed that there were significant differences among the diurnal temperature regimes (16/8 ºC vs. 20/12ºC, and 24/16 ºC) in their effect on flowering of the accessions. Significant difference was detected between photoperiod-sensitive and day-neutral; photoperiod-sensitive and intermediate; and day-neutral and intermediate accessions for their flowering responses.

Origin of the genotypes played a significant role in the relative earliness of the genotypes. Those with the tropical origin flowered relatively earlier compared to those cultivars developed in the Crop Development Centre, University of Saskatchewan. A previous report indicated that chickpeas of Indian origin were far more responsive to temperature than those from Mediterranean type climates (Berger et al. 2011). Because crop duration is affected by the onset of flowering in chickpea accessions, the effect of temperature on flowering revealed a very significant effect on crop duration in accessions. Breeding of chickpea cultivars for specific adaptation should focus on matching phenology with precipitation of the region as well (Vadez et al., 2013). In western Canada, the growing duration of a crop must closely match the available growing season. Because chickpea is a highly indeterminate crop, excess moisture boosts tremendous vegetative growth (Anbessa

et al., 2007*b*). Thus, earlier flowering and photoperiod-insensitive genotypes can be sought for adaptation in the Canadian prairies.

Knowledge of the timing when plants become receptive to inductive photoperiods and the effects of photoperiod regimes on the flowering pattern of the crop would allow producers to appropriately schedule production calendars to reduce crop production time with the necessity to reduce crop yield and quality. The presence of weak photoperiod-sensitivity in soybean cultivars adapted to high latitudes facilitated earlier flower initiation under long-day conditions in a narrow frost-free seasons (Tsubokura et al., 2013).

Short-day treatments delayed flowering as compared to long-days during the first few weeks after emergence. For the rest of the genotypes, the photoperiod sensitive phase was identified. The photoperiod-sensitive phase extends beyond flower initiation into full flower development. The photoperiod-sensitive phase was longer under short days compared to under long days and the duration of this phase is genotype specific. Thus, we have concluded that the phenology of chickpea genotypes from emergence to first flower can be divided into three phases: a photoperiod-insensitive pre-inductive phase, a photoperiod-sensitive inductive phase and a photoperiod-insensitive post-inductive phase under both long and short days. In the day-neutral accessions however, there was no significant difference in days to flowering under long days and short days and subsequent transfers.

Late flowering leads to a longer vegetative growth period under ideal growing conditions thereby promoting the accumulation and allocation of more resources to seed production, whereas early flowering is desired in environments with a short or unpredictable growing season (Simpson and Dean, 2002). The compromise between resource accumulation and stress avoidance is also of primary importance for crop yield and quality, and the identification of molecular variation associated with flowering time is a key step to selecting varieties adapted to different latitudes and cropping seasons.

Information on modes of inheritance of time to flowering and ascochyta blight resistance in chickpea were reported by different authors. Similarly, QTL for resistance to ascochyta blight and time to flowering were also inconsistent as a result of differences in the genetic materials used and evaluation techniques by the respective researchers. Research conducted under growth chamber and field conditions to evaluate CP-RIL-1 revealed the existence of wide variability in the RIL lines and the two parents for their reaction to ascochyta blight. Ascochyta blight scores in the field and growth chamber conditions were positively correlated. Similar values of broad sense hertitability were detected for days to flowering under both field and growth chamber conditions.

Wide variability was also observed in the RILs and the parents for their flowering response both under growth chamber and field conditions. Our findings indicated the existence of positive and significant association between number of days to flowering and number of nodes of first flower under short days. Time to flowering under growth chamber and field conditions were positively correlated.

There was significant and negative correlation between the number of days to flowering and ascochyta blight resistance of the CP-RIL-1 at all the three test locations (Elrose, Moose Jaw and Floral) in 2012. In general, the early flowering and maturing lines were highly susceptible to ascochyta bight, while those flowering and maturing late were more resistant to ascochyta blight. This research also noted negative correlation between photoperiod sensitivity and ascochyta blight resistance, which hinders simultaneous improvement of these traits in chickpea. Selection over years for early flowering, photoperiod insensitivity and ascochyta blight resistance might lead to a considerable correlated response of these traits.

In spite of the large collection of germplasms at ICRISAT and ICARDA together with national genetic improvement programs, the narrow genetic base of chickpea continues

to be a widespread concern (Upadhyaya, et al. 2008). Currently, with the discovery of many genomic markers, it is possible to utilize molecular markers in chickpea for identifying genetically diverse germplasm with beneficial traits for use in crop improvement programs. Genetic improvement programmes can be enhanced by the use of molecular genetic information for introgression, genotype building and recurrent selection programmes (Dekkers and Hospital, 2002; Xu and Crouch, 2008). Incorporation of genes for early maturity and photoperiod insensitivity into unadapted germplasm is a desired target in several crops such as spring wheat (Dyck et al., 2004) and common bean (Singh, 2001). Upadhyaya, et al. (2008) suggested utilization of the highly polymorphic markers and genetically diverse chickpea accessions with beneficial traits from the Mediterranean and African regions in genomics and breeding of the crop. Thus, to attain appropriate level of maturity in chickpea for adaptation in western Canada, further discovery of genes and regulatory pathways controlling flowering and maturity time could be exploited. In conclusion, the markers bordering the QTLs for the traits explored in this thesis are likely to be useful for selecting favorable lines by markers assisted selection.

7.1 Conclusions

Variability in flowering response under different photoperiods was detected among the chickpea accessions representing both, desi and kabuli market classes, collected from wide environments. A negative and significant correlation between days to flowering and the cumulative photo-thermal unit as well as linear regression with both cumulative thermal unit and photo-thermal unit was detected in the chickpea accessions. The origin of the chickpea accessions had a significant effect on flowering responses in response to a combination of photoperiod and temperature regimes; those accessions originating in the tropical environments were early flowering and were identified as less sensitive to changes in photoperiod, whereas those accessions originating from an adapted to the temperate environments were late flowering and highly sensitive to changes in photoperiod.

Photoperiod sensitive phases were identified in chickpea accessions using a reciprocal transfer experiment from short to long day photoperiod chambers. Based on these experiments, the phenology of chickpea can be divided into three phases namely the photoperiod-insensitive pre-inductive phase, the photoperiod-sensitive inductive phase, and the photoperiod-insensitive post-inductive phase under long as well as short-day photoperiod conditions. The significant and negative correlation between days to flowering and ascochyta blight resistance, and between photoperiod sensitivity and ascochyta blight resistance revealed that earlier flowering and day-neutrality are associated with susceptibility to ascochyta blight. Earliness and ascochyta blight resistance are important breeding objectives in chickpea in western Canada; however, the significant negative correlation between the traits causes difficulties in achieving both goals simultaneously.

7.2 Future Research

Days to flowering was reduced under long day photoperiods compared with short day photoperiods. Higher temperature regimes and longer photoperiod treatments also reduced time to flowering in chickpea accessions. This experiment was conducted using a factorial combination of three temperature regimes and two photoperiods. It is anticipated that the results from this study would significantly contribute towards the understanding of flowering time under two contrasting photoperiod combined with a range of temperature regimes. Resource limitation was the key determinant to conducting indoor experiments of such kind over a wider range of photoperiod and temperature regimes. However, for further elucidation of this result, evaluation of chickpea accessions under wider photoperiod and temperature regimes need be conducted to identify chickpea accessions/genotypes with early flowering response to changes in photoperiod and temperature, which in turn would assist the substantial improvement of earliness in western Canada.

A photoperiod sensitivity phase was identified in chickpea accessions by reciprocal transfer of the plants from long days (inductive) to short days (less-inductive) photoperiod treatment based on a customized time of transfer. It is also anticipated that findings from the current study can provide insight into the identification of three photoperiod response phases in chickpea accessions with pronounced response to changes in photoperiod. Further, this study was conducted under a controlled temperature and photoperiod in a growth chamber combined with an analysis presented here. In order to further elucidate the results from this study, ranges of time of reciprocal transfers should be adjusted to less than the ranges of days of transfers used in this study. Further experiments could be conducted to determine what genes are involved in the expression of flowering during each of the three response phases.

Recently, the chickpea reference genome sequence has become available assisting in sequence analysis in the vicinity of QTLs to allow selection of linked markers across a broad range of germplasm sources (Stephens et al. 2014). The identification of candidate genes and elucidation of their role could be facilitated by combining QTL analysis with the available sequence information of CDC Frontier (Varshney et al., 2005; Varshney et al. 2013).

Several QTLs identified in this study for time to flowering and maturity, morphological, and disease resistance traits have widened our understanding of the genomic regions associated with these traits. However, because a single QTL may represent many genes it is important to identify specific and individual candidate gene sequences that may account for the identified QTL effects (Flowers, 2004).

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APPENDIX I. List of chickpea accessions and their country of origin evaluated for response to days to flowering under long and short-day photoperiod treatments.

No	Origin	No. of accessions	List of accessions
1	Afghanistan	7	ILC 1329, ILC 1493, ILC 1539, ILC 1595, ILC 1685,ILC 1687, ICC 8855
2	Algeria	2	ILC 606, ICC 2210
3	Armenia	1	ILC182
4	CDC	7	CDC Corinne, CDC Luna, CP 71, CDC Xena, CP55, CDC Frontier
5	Cyprus	2	ICC 3325, ICC 12328
6	Egypt	1	ILC531
7	Ethiopia	5	ICC 1386, ICC 8607, ICC 8621, ICC13892, ICC 14077
8	Greece	1	ILC 153
9	ICARDA, Syria	12	FLIP 98-135C, FLIP 95-55C, FLIP 97-657C, FLIP 98-142C, FLIP 94-090C, FLIP 97-95C, FLIP 82-150C, FLIP 91-24C, FLIP 84-153, FLIP 88-85, ICC 15802
10	ICRISAT, India	17	ICC 12968, ICCV 96029, ILC 243, ILC 272, ICC 1180, ICC 1205, ICC 1882, ICC 1915, ICC 5879, ICC 6279, ICC 8350, ICC 8950, ICC 11378, ICC 12928, ICC 13077, ICC 15996, ICC 16903
11	Iran	22	ILC 659, ILC 1153, ILC 1264, ILC 1269, ILC 2003, ICC 2629, ICC 2720, ICC 2919, ICC 2990, ICC 3761, ICC 4814, ICC 6811, ICC 6877, ICC 7819, ICC 8058, ICC 13187, ICC 13357, ICC 13441, ICC 13523, ICC 13524, ICC 13764, ICC 15294
12	Iraq	1	ILC 234
13	Israel	1	ICC 7571
14	Kazakhstan	1	ILC 9757
15	Malawi	1	ICC 16261
16	Morocco	2	ILC 3352, ICC 15435
17	Russian Federation	5	Pch 5, ICC 6263, ICC 6306, ICC 7668, ICC 11284
18	Sudan	1	ILC 205
19	Tajikistan	1	ILC 7259
20	Turkey	5	ILC 459, ILC 1300, ILC 1309, ICC 7184, ICC 8261
21	Unknown	5	ICC 9402, ICC 9586, ICC 9744, ICC 9848, ICC 9895

No	Origin	No. of accessions	List of accessions
22	Bulgaria	1	Amit
	Total	100	

APPENDIX II. Category of photoperiod-insensitive (day-neutral), intermediate, and photoperiod-sensitive categories of chickpea accession and country and/or institute of origin.

Category	No	Accessions	PS	Origin
Day-neutral	1	FLIP 98-142C	10	ICARDA
	3	ICCV 96029, ICC 12968, ICC 8350	1-8	ICRISAT
Intermediate	4	ICC 8855, ILC 1329, ILC 1539, ILC 1595	21-33	Afghanistan
	1	CDC Xena	30	CDC
	1	ICC 3325	27	Cyprus
	1	ILC531	15	Egypt
	2	ICC 8621, ICC 14077, ICC 13892, ICC 13863	20-36	Ethiopia
	2	FLIP 98-135C, FLIP 97-95C	16-31	ICARDA
	9	ICC 15996, ICC 1882, ICC 6279, ILC 243, ICC 5879, ICC 1180, ICC 13077, ICC 1915, ICC 11378	19-39	ICRISAT
	13	ICC 2919, ICC 6877, ILC 659, ICC 2990, ICC 13524, ICC 2629, ICC 8058, ICC 4814, ICC 7819, ICC 3761, ICC 13523, ICC 13764, ICC 15294	17-40	Iran
	1	ILC234	35	Iraq
	1	ILC 9757	31	Kazakhstan
	2	ICC 15435, ILC 3352	20-33	Morocco
	2	ICC 6263, ICC 7668	33-34	Russia
	1	ILC 205	26	Sudan
	3	ILC 459, ILC 1300, ICC 7184	33-40	Turkey
	2	ICC 9895, ICC 9848	20-37	unknown
	1	ILC188	38	Uzbekistan
Photoperiod sensitive	3	ILC 1687, ILC 1685, ILC 1493	41-47	Afghanistan
	2	ILC 606, ICC 2210	47-66	Algeria
	1	ILC 182	53	Armenia
	5	CDC Luna, CP 71, CDC Corinne, CDC Frontier, CP55	44-62	CDC
	1	Amit	44-62	Bulgaria
	1	ICC 12328	41	Cyprus

Category	No	Accessions	PS	Origin
	1	ICC 8607	43	Ethiopia
	1	ILC 153	43	Greece
	8	FLIP 97-657C, FLIP 88-85, FLIP 82-150C, FLIP 91-24C, FLIP 84-153, FLIP 94-090C, FLIP 95-55C, ICC 15802	44-62	ICARDA
	5	ICC 16903, ILC272, ICC 12928, ICC 8950, ICC 1205	46-56	ICRISAT
	9	ILC 1269, ILC 1264, ICC 6811, ICC 13357, ILC 2003, ICC 13441, ICC 2720, ICC 13187, ILC 1153	42-65	Iran
	1	ICC 7571	48	Israel
	3	Pch 5, ICC 11284, ICC 6306,	50-60	Russia
	1	ILC 7259	61	Tajikistan
	2	ICC 8261, ILC 1309	43-53	Turkey
	3	ICC 9402, ICC 9744, ICC 9586	44-56	Unknown

* PS= Photoperiod sensitivity, derived as a number of days to flowering delayed under short days compared to long days. The intervals indicate the range of photoperiod sensitivity of individual accession collected from the respective countries.

Appendix III. Thermal units and photo-thermal units required for first flower in chickpea accessions evaluated under short and long day in growth chamber conditions.

Accessions	Long days			Short days			PS		
	mean dtf	TU (20)	PhtU (14.7)	mean dtf	TU (18.5)	PhtU (9.2)	PS	TU (20)	PhtU (18.5)
ILC153	40	800	588	83	1536	764	43	860	796
ILC 182	46	920	676	99	1832	911	53	1060	981
ILC 188	41	820	603	79	1462	727	38	760	703
ILC 205	39	778	572	65	1196	595	26	516	477
ILC 234	30	590	434	65	1209	601	35	717	663
ILC 243	33	660	485	61	1125	560	28	557	515
ILC 272	43	860	632	91	1690	840	48	967	894
ILC 459	37	744	547	70	1301	647	33	663	613
ILC 531	36	723	532	51	950	472	15	303	281
ILC 606	34	672	494	80	1480	736	46	928	858
ILC 659	29	573	421	54	1005	500	25	513	475
ILC 1153	41	828	609	106	1961	975	65	1292	1195
ILC 1264	31	612	450	75	1392	692	44	893	826
ILC 1269	31	617	453	73	1351	672	42	843	780
ILC1300	40	790	581	76	1400	696	36	723	669
ILC 1309	38	751	552	91	1677	834	53	1063	983
ILC 1329	34	677	497	61	1129	561	27	543	503
ILC 1493	33	663	488	80	1486	739	47	943	873
ILC 1539	25	503	370	53	987	491	28	563	521
ILC 1595	28	563	414	62	1138	566	33	667	617
ILC 1685	38	760	559	83	1536	764	45	900	833
ILC 1687	33	657	483	74	1363	678	41	817	755
ILC 2003	31	620	456	80	1488	740	49	988	914
ILC 3352	32	643	473	65	1203	598	33	657	607
ILC 7259	34	677	497	95	1758	874	61	1223	1132
ILC 9757	34	687	505	66	1212	603	31	623	577

Accessions	Long days			Short days			PS		
	mean dtf	TU (20)	PhtU (14.7)	mean dtf	TU (18.5)	PhtU (9.2)	PS	TU (20)	PhtU (18.5)
ICC 1180	29	573	421	66	1215	604	37	740	685
ICC 1205	32	633	466	88	1622	807	56	1120	1036
ICC 1882	36	720	529	55	1024	509	19	387	358
ICC 1915	44	887	652	83	1543	767	39	782	723
ICC 2210	39	780	573	99	1832	911	60	1200	1110
ICC 2629	30	590	434	58	1073	534	28	570	527
ICC 2720	37	730	537	89	1647	819	52	1050	971
ICC 2919	42	830	610	58	1079	537	17	337	311
ICC 2990	38	758	557	65	1203	598	27	542	502
ICC 3325	35	690	507	62	1141	567	27	543	503
ICC 3761	27	540	397	60	1110	552	33	660	611
ICC 4814	25	503	370	57	1061	527	32	643	595
ICC 5879	28	277	203	62	1147	570	34	963	891
ICC 6263	35	690	507	68	1264	629	33	677	626
ICC 6279	38	767	564	58	1061	527	19	380	351
ICC 6306	35	697	512	94	1739	865	59	1183	1095
ICC 6811	38	753	554	85	1573	782	47	947	876
ICC 6877	39	780	573	62	1138	566	23	450	416
ICC 7184	47	940	691	87	1610	800	40	800	740
ICC 7571	32	640	470	80	1474	733	48	953	882
ICC 7668	41	820	603	75	1378	685	34	670	620
ICC 7819	47	940	691	79	1462	727	32	640	592
ICC 8058	43	860	632	71	1318	656	28	565	523
ICC 8261	43	860	632	86	1585	788	43	853	789
ICC 8350	36	720	529	44	814	405	8	160	148
ICC 8607	27	533	392	70	1289	641	43	860	796
ICC 8621	33	653	480	53	981	488	20	407	376
ICC 8855	25	497	365	46	851	423	21	423	392
ICC 8950	26	520	382	77	1431	711	51	1027	950

Accessions	Long days			Short days			PS		
	mean dtf	TU (20)	PhtU (14.7)	mean dtf	TU (18.5)	PhtU (9.2)	PS	TU (20)	PhtU (18.5)
ICC 9402	45	900	662	89	1653	822	44	887	820
ICC 9586	36	717	527	92	1702	846	56	1123	1039
ICC 9744	24	473	348	68	1264	629	45	893	826
ICC 9848	43	860	632	80	1480	736	37	740	685
ICC 9895	34	683	502	54	1005	500	20	403	373
ICC 11284	32	640	470	82	1508	750	50	990	916
ICC 11378	33	667	490	73	1344	669	39	787	728
ICC 12328	38	750	551	78	1443	718	41	810	749
ICC 12928	37	730	537	85	1566	779	48	963	891
ICC 12968	33	657	483	31	574	285	2	37	34
ICC 13077	45	894	657	83	1536	764	38	766	709
ICC 13187	28	553	407	92	1708	849	64	1293	1196
ICC 13357	30	597	439	78	1449	721	48	970	897
ICC 13441	35	703	517	84	1559	775	49	982	908
ICC 13523	29	576	423	62	1138	566	33	654	605
ICC 13524	28	560	412	55	1011	503	27	533	493
ICC 13764	36	717	527	69	1277	635	33	663	614
ICC 13863	32	633	466	68	1258	626	36	727	672
ICC 13892	31	627	461	59	1092	543	28	553	512
ICC 14077	32	630	463	52	962	478	20	410	379
ICC 15294	37	730	537	77	1415	704	40	800	740
ICC 15435	32	630	463	52	953	474	20	400	370
ICC 15802	30	590	434	NR	NR	NR	NR	NR	NR
ICC 15996	33	657	483	62	1141	567	29	577	533
ICC 16261	NR	NR	NR	67	1247	620	NR	NR	NR
ICC 16903	28	560	412	74	1372	682	46	923	854
FLIP 98-1	30	597	439	46	851	423	16	323	299
CP 55	43	860	632	97	1788	889	54	1073	993
Amit	43	860	632	109	2017	1003	66	1320	1221

Accessions	Long days			Short days			PS		
	mean dtf	TU (20)	PhtU (14.7)	mean dtf	TU (18.5)	PhtU (9.2)	PS	TU (20)	PhtU (18.5)
CDC Frontier	39	787	578	109	2017	1003	70	1393	1289
CDC Corinne	44	875	643	106	1961	975	62	1245	1151
CDC Lum	42	842	619	89	1642	817	47	933	863
ICCV 96029	29	580	426	30	561	279	1	27	25
CP 71	39	777	571	92	1702	846	53	1063	984
CDC Xena	37	740	544	67	1240	616	30	600	555
FLIP 95-55C	38	757	556	100	1850	920	62	1243	1150
FLIP 97-657C	40	803	590	84	1549	771	44	872	806
Pch 5	36	710	522	95	1761	876	60	1193	1104
FLIP 98-142C	28	567	417	38	703	350	10	200	185
FLIP 94-090C	37	733	539	98	1813	902	61	1227	1135
FLIP 97-95C	44	883	649	75	1388	690	31	617	570
FLIP 82-150C	38	759	558	93	1724	857	55	1104	1021
FLIP 91-24C	46	920	676	101	1869	929	55	1100	1018
FLIP 84-153	40	800	588	99	1832	911	59	1180	1092
FLIP-88-85	46	920	676	96	1770	880	50	993	919

TU= Thermal units; PhtU = Photo-thermal units; Dtf = days to flowering; PS= Photoperiod sensitivity; NR= not recorded.

APPENDIX IV. Photo-thermal conditions used for the determination of flowering response of chickpea accessions to temperature and photoperiod.

Photoperiod (hours)		Temperature (˚C)			Light intensity ($\mu mol\ m^{-2}s^{-1}$)
Day	Night	Day	Night	Mean	
16	8	24	16	20	370
16	8	20	12	16	370
16	8	16	8	12	370
10	14	24	16	20	370
10	14	20	12	16	370
10	14	16	8	12	370

APPENDIX V. Typical planting, flowering and harvesting times and photoperiod during flowering of chickpea in India and Canada.

Regions	Planting time	Flowering time	Photoperiod (in hours) during flowering	Harvesting time
South India (15.5 to 17.4˚)	November	December	11.2	March
North India (26.5 to 30. 9˚)	October	January	10.5	March
Western Canada	May	July	16	September

APPENDIX VI. Total number of time replicates, replications and dates of plantings, first and second ratings of ascochyta blight reaction of CP-RIL-1 evaluated in greenhouse for their response to ascochyta blight (isolate Ar-170) in 2011.

Time replicates	Number of replications	Planting	Rating 1	Rating 2
1	2	June, 1	June, 22	June, 29
2	2	June, 21	July, 15	July, 22
3	4	July, 18	August, 8	August, 15
4	4	August, 14	Sept, 4	September, 10

Appendix Figures

Figure I. Effect of temperature on flowering and crop duration in chickpea accession (ICC 8621). The accession was evaluated for flowering response under short-days combined with mean temperatures of and 16/8 ˚C, 20/12 ˚C, and 24/16 ˚C, day and night, respectively.

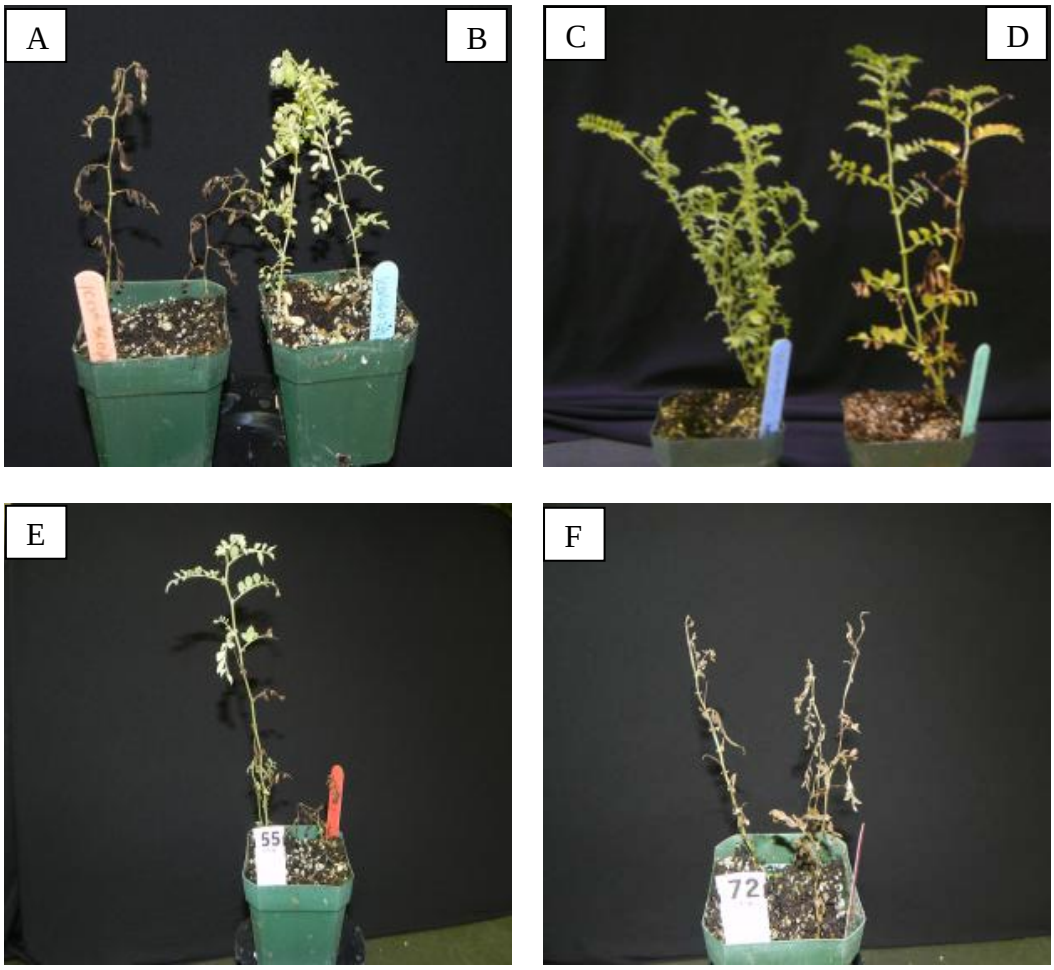

Figure II. Reaction of the two parents (ICCV 96029 and CDC Frontier) and other lines to Ar-170 inoculum and water treatment.
A = ICCV 96029 the highly susceptible parent inoculated with the Ar-170 inoculum
B = ICCV 96029 treated with water as control
C= CDC Frontier the moderately tolerant parent treated with water
D = CDC Frontier treated with Ar-170 inoculum
E = Entry 55 (IC-Fr-94)
F = Entry 72 (IC-Fr-142)
The pictures were taken 3 weeks after inoculation.